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MODULATION OF REFLEXES DURING LOCOMOTION

by

SOPHIE JEANNINE DE SERRES

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in partial fulfillment of the requirements for the degree of

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Sophie De Serres

Sophie Jeannine De Serres
12051 boul. Taylor
Montréal, Québec
H3M 3C8

October 1996

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled MODULATION OF REFLEXES DURING LOCOMOTION submitted by SOPHIE JEANNINE DE SERRES in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

Dr. Richard B. Stein

Dr. Peter B.C. Matthews

Dr. Jaynie F. Yang

Dr. Keir G. Pearson

Dr. Arthur Prochazka

Oct. 1, 1996
date

ABSTRACT

Responses to either electrical or mechanical stimuli are highly modulated according to the task performed (standing, walking or running) or to the phase of the rhythmic movement in which the stimuli are applied. However, the mechanisms responsible for modulating reflexes during locomotion are not all yet identified.

A reversal in the sign of a cutaneous reflex during human walking was studied using single motor unit recordings. The results support the hypothesis that there are parallel excitatory and inhibitory pathways from cutaneous afferents to single motoneurones of the tibialis anterior muscle. A shift in balance between the two pathways as a function of the step cycle most likely generates the reflex reversal observed.

Experiments performed on decerebrate cats allowed the study of some mechanisms involved in regulating the stretch reflex amplitude during tonic muscle contractions and, after spinalization, during locomotion. Spindle afferent activity was recorded while the soleus muscle was sinusoidally stretched. High spindle sensitivity in resting decerebrate and spinal cats, due to predominant dynamic fusimotor drive, decreased abruptly at the transition from resting to tonic or phasic (walking) activity, attributable to either an increase in static γ-drive or spindle unloading due to α without γ input.

Reflex force and electromyographic responses to stretch were measured in the triceps surae muscles with pulse, ramp-and-hold and sinusoidal perturbations. Opposite relationships between reflex amplitude and background force were observed when comparing tonically to phasically active muscles: the reflex decreased with stronger tonic contractions while it

paralleled the contraction level during walking. Despite a decrease in spindle sensitivity, the reflex reduction was much greater during locomotion than for tonic contractions, suggesting that other mechanisms are involved in reflex attenuation.

The reflex loop gain, computed with sinusoidal and pulse stretch responses, mirrored the reflex behaviour seen in the two tasks. A substantial difference in the reflex gain, between the two stretches, highlighted the nonlinear characteristic of this reflex system. An augmentation in the gain with a decrease in external load stiffness during tonic contractions, but not in walking, further emphasized that segmental control mechanisms play an important role in the reflex reduction during locomotion.

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LIST OF ABBREVIATIONS

A/D = analog to digital

ANOVA = analysis of variance

cm = centimetre

CO_2 = carbon dioxyde

CP = common peroneal

CPG = central pattern generator

DC = direct current

deg = degree

EMG = electromyography

F = force

F_r = reflex force

F_t = total force

FRA = flexion reflex activation

G = gain

γ_d = dynamic gamma

γ_s = static gamma

Hz = Hertz

imp/s = impulses per second

K = stiffness

K_e = external load stiffness

K_i = intrinsic stiffness

K_p = parallel component stiffness

K_s = series elastic component stiffness

kg = kilogram

kHz = kiloHertz

L = length

L-DOPA, (DOPA) = Levodopa, L-3,4-dihydroxylphenylalanine

L_0 = initial length

M = gain magnitude

mg = milligram

min = minute

ml = millilitre

MLR = mesencephalic locomotor region

mm = millimetre

mmHg = millimetre of mercury

mod. = modulation

ms = millisecond

mV = milliVolts

MVC = maximal voluntary contraction

N = Newton

NA = noradrenergic

O_2 = oxygen

PSP = postsynaptic potential

PSTH = post stretch triggered histogram

R = reflex response

RMS = root mean square

s = second

SD = standard deviation

SE = standard error

σ = variance

TA = tibialis anterior

TS = triceps surae

VAF = variance accounted for

X-ext = crossed extension

CHAPTER 1

GENERAL INTRODUCTION

> *"Reflex responses, the knee jerk, the withdrawal of a hand from a hot object, or coughing are the simplest motor behaviors and are least affected by voluntary controls. Reflexes are rapid, somewhat stereotyped, and involuntary responses that are usually controlled in a graded way by the eliciting stimulus."*
>
> - Kandel, Schwartz & Jessell (1991)

This statement constitutes a rather general definition of a reflex. Considering the substantial number of studies concerning segmental reflex modulation, published in the past three decades, reflexes are certainly not just simple motor behaviours and stereotyped responses, graded only by the eliciting stimulus. They display a variety of behaviours whether they are made of polysynaptic, disynaptic, or even monosynaptic loops.

1.1 MODULATION OF REFLEXES DURING LOCOMOTION

Already in the early 1900s, reflex responses were shown to vary markedly with postural attitude (Sherrington, 1900; Graham Brown, 1911, 1914; Graham Brown & Sherrington, 1912; Ranson & Hinsey, 1930). More recently, many studies have shown that responses to either electrical or mechanical stimuli can be highly modulated according to the task performed (standing, walking or running) or to the phase of the rhythmic movement

in which the stimuli are applied. Cutaneous stimulation applied to a hindlimb of acute spinal cats may evoke in the other hindlimb an extension or a flexion response depending whether it is respectively flexed or extended at the time of stimulation (Rossignol & Gauthier, 1980). Also, studies in intact cats (Duysens & Stein, 1978; Forssberg, 1979; Wand, Prochazka & Sontag, 1980; Duysens, Loeb & Weston, 1980), thalamic cats (Duysens & Pearson, 1976) and spinalized cats (Grillner, 1973; Forssberg, Grillner & Rossignol, 1975, 1977; Grillner & Rossignol, 1978a; Andersson, Forssberg, Grillner & Lindquist, 1978) have shown that a cutaneous stimulus applied during the step cycle generates two different effects: excitation of the extensors in the stance phase and excitation of the flexors in the swing phase. These different effects appear to be functionally appropriate in permitting the cat to maintain a stable posture or a forward progression in response to an unexpected sensory stimulus during walking. Also, responses following a mechanical stimulation (stretch reflex as in Akazawa, Aldridge, Steeves & Stein, 1982) or an electrical stimulation (monosynaptic H-reflex as in Duenas, Loeb & Marks, 1990) of the primary afferents were shown to be modulated throughout the cat step cycle.

In humans, the monosynaptic H-reflex is modified quickly for different tasks such as standing, walking and running (Stein & Capaday, 1988) and is also highly modulated as a function of time during the walking cycle (Capaday & Stein, 1986, 1987; Crenna & Frigo, 1987; Yang & Whelan, 1993). Similarly, the mechanically induced stretch reflex was modulated during human walking and was estimated to contribute substantially to the activation of the soleus muscle particularly during the early part of the stance phase (Yang, Stein & James, 1991). Others have also reported on phase-dependent modulation of cutaneous reflexes in human locomotion (Crenna & Frigo, 1984; Bélanger & Patla, 1987;

Duysens, Tax, van der Doelen, Trippel & Dietz, 1991). These studies have shown a variation in the amplitude of the reflex response during walking. Sometimes this modulation can affect the sign of the response (excitatory or inhibitory). Indeed, a phase-dependent reflex-reversal was observed in the tibialis anterior muscle during human walking in response to a cutaneous stimulation (Yang & Stein, 1990; Duysens, Trippel, Horstmann & Dietz, 1990). When the stimulus was applied during the middle of the swing phase, there was an excitatory response at a medium latency. When the stimulus was applied during the transition from swing to stance, there was an inhibitory response at the same latency.

1.2 SENSORY FEEDBACK AND RHYTHMIC MOVEMENTS

Such a large variety of reflex behaviours gave rise to two important questions about the role of sensory inputs during rhythmic movements, particularly during locomotion. (1) Are inputs from a variety of sensory receptors (skin, muscle, joint) necessary for the generation of cyclic movements? and, (2) Are sensory inputs important in regulating the pattern of muscle activation in rhythmic movements?

RHYTHMIC MOVEMENT GENERATION. Despite the fact that an answer to the first question had been proposed over 80 years ago:

> *"Cutaneous afferents of the limb are inessential for the reflex step. The reflex step is as regards the afferents of the stepping limb itself essentially a proprioceptive reflex. Hence the normal stepping (cat, dog) is curiously little disturbed by denervation of the foot or anaesthesia of the limb-surface."*
>
> - C.S. Sherrington (1910),

the topic has been debated until recently (for review see Delcomyn, 1980). It is now clear that an acutely deafferented limb can produce the basic sequence of muscle activity, including complex EMG patterns (Grillner & Zangger, 1984). Also some experiments have shown that in the mammal and lower vertebrates, most features of the motor pattern underlying locomotion can be elicited after a bilateral deafferentation (Taub, 1976; Grillner & Wallén, 1985). Moreover, in a decorticated and paralyzed cat, a spontaneous and well-organized rhythmic locomotor activation pattern can be recorded in the nerves of the hindlimbs (Perret, 1976; Perret & Cabelguen, 1980; Perret, 1983). Similar observations were reported in other paralyzed cat preparations ('fictive' locomotion): in thalamic cats (Bayev, 1978a), in decerebrate cats in which locomotion was induced by stimulation of the mesencephalic locomotor region (MLR, Jordan, Pratt & Menzies, 1979; Arshavsky, Orlovsky, Pavlova & Popova, 1986), and finally, in acute spinal cats (Jankowska, Jukes, Lund & Lundberg, 1967; Grillner & Zangger, 1979). Rhythmic patterns are generated in the absence of afferent feedback not only for locomotion but also for other types of cyclic movement such as mastication and respiration (for review see Rossignol, Lund & Drew, 1988).

The fact that these rhythmical processes do occur in the absence of sensory feedback and supraspinal inputs (spinalized animal) certainly suggests that they are generated in the central nervous system by sets of specially wired neuronal networks which have been called central pattern generators (CPGs). The term CPG started to be used only recently (Grillner & Zangger, 1975), even though Graham Brown (1911) had already pointed out that rhythmic contractions of leg muscles, similar to those that occur during walking, could be induced immediately following transection of the spinal cord even in animals in which all inputs from sensory nerves in the legs had been eliminated. This discovery had led Graham Brown to

propose that mechanisms located entirely within the spinal cord are responsible for generating the basic rhythm for stepping in each leg.

RHYTHMIC MOVEMENT REGULATION. Even if it was shown that sensory feedback from muscle or skin receptors is not necessary for generating a rhythmic pattern of muscle activation such as locomotion, it remains a major factor in the regulation of these rhythmic movements. Some of the most important roles of sensory feedback are in establishing details of the temporal order of motor activity; in controlling transitions from one phase of a movement to another; and in reinforcing ongoing motor activity (Pearson, 1993). It is now well acknowledged that muscle and cutaneous reflexes are not imperative for the generation of the locomotor rhythm, and that they play an important role in the regulation of the activation pattern. The majority of the studies performed in the last twenty years have mainly focused on determining which neural mechanisms are involved in producing the reflex modulation, and on identifying more precisely the specific role of a cutaneous or muscle reflex in shaping the pattern of muscle activation during rhythmic movements.

1.3 NEURAL MECHANISMS FOR REFLEX MODULATION

The neural mechanisms which are responsible for modulating reflexes during locomotion are not all identified yet. Again, there are two important aspects to consider in an attempt to understand the control of reflex modulation: (1) Which supraspinal and/or segmental structures exert an influence on the reflex response for different tasks and specific

phases of a cyclic movement? (2) Which component(s) of the reflex pathway is(are) the receiving site(s) of this external influence?

As mentioned earlier, modulation of cutaneous reflexes during locomotion has been reported for a broad group of animal preparations (from intact to spinal), as well as in humans (refer to section 1.1). These results strongly suggest a role for the spinal structures, particularly the CPG, in affecting the response to a skin stimulus. However, it is important to specify that the fact that the reflex modulation is mediated via segmental structures does not discard the possibility of any supraspinal control in the fine tuning of these reflex responses. Effectively, supraspinal structures were shown to send signals to the spinal cord through vestibulo- and rubro-spinal neurones (Grillner & Lund, 1968; Grillner & Hongo, 1972; Hongo, Jankowska & Lundberg, 1972), and via the pyramidal tract fibres (Pinter, Burke, O'Donovan & Dum, 1982), with targets on motoneurones as well as interneurones mediating spinal reflexes.

On the other hand, the monosynaptic reflex from muscle primary afferents was seen to vary within the step cycle in humans (H-reflex: Capaday & Stein, 1986, 1987a; Crenna & Frigo, 1987, Dietz, Faist & Pierrot-Deseilligny, 1990; stretch reflex: Dietz, Discher, Faist & Trippel, 1990) and in decerebrate cats (both stretch and H reflexes: Akazawa *et al.* 1982). No study so far has looked at this monosynaptic reflex in the spinal animal, in order to determine if the source of its modulation is located in the spinal cord. However, Schomburg and Behrends (1978) recorded Ia excitatory postsynaptic potentials (PSPs) in some motoneurones during fictive locomotion in paralyzed spinal animals. They showed that there was some modulation of monosynaptic excitatory PSP amplitude and that the Ia-evoked polysynaptic excitatory PSPs became more prominent during the phase of locomotion where

the particular motoneurone was active. Also, it was shown that the locomotor rhythm elicited by Clonidine, a noradrenergic agonist, is also accompanied by presynaptic activity in primary afferents in the spinal cat (Gossard, Cabelguen & Rossignol, 1991). These results suggest that the stretch reflex elicited after spinalization in walking cats should display a similar modulation to that observed in the decerebrate cats. This issue is considered in the present work since stretches to the triceps surae muscles were applied in spinalized cats with Clonidine- or DOPA-induced locomotor rhythm (Chapters 4 and 5).

Rossignol *et al.* (1988) and Sillar (1991) discussed extensively the different levels in the reflex pathway where certain structures, external to the reflex loop, can act to produce the modulation. The mechanisms to be considered in a monosynaptic reflex pathway are (1) a change in the level of excitability of the motoneurones, (2) a variation in the efficiency of the synaptic transmission between the afferent and the motoneurone, and (3) a change in the sensitivity of the sensory receptor. When disynaptic and polysynaptic pathways are involved, this external influence can also be occurring at the interneuronal level. In the following subsections, these possibilities are treated, emphasizing specific examples related to the studies presented in the following chapters.

1.3.1 Excitability level of α-motoneurones

Electrical stimulation of the posterior tibial nerve in the popliteal fossa evokes a contraction of the calf muscles. This was first shown at the beginning of the century and it was demonstrated that the response observed consisted of a short-latency and/or a delayed activation of the motor units of the triceps surae muscle. The reflex-elicited contraction of the calf muscles was due to activation of group Ia fibres from spindles,

and was later called the Hoffmann reflex, or "H-reflex", whereas the direct motor response was called the "M-response".

The H-reflex methodology has been extensively used for assessing spinal reflex excitability, for studying stimulus-induced and/or task-related changes in the excitability of human motoneurones (for review see Schieppati, 1987). For example, the amplitude of the H response of the human soleus muscle was measured during standing, walking and running, in order to compare the changes in spinal reflex excitability between the three tasks (Stein & Capaday, 1988). The results demonstrated a decrease in the H-reflex amplitude when comparing walking to standing, as well as when comparing running to walking. It is important to note that the comparisons were made for the same background level of EMG activity, which ruled out the possibility that the observed differences were due to a change in the level of motor activity of the soleus muscle. Moreover, the M-response was measured throughout the experimental protocol and the intensity of the electrical stimulus was adjusted so that a constant stimulus was applied to the tibial nerve, independently of the task performed by the subject.

However, data obtained in the mesencephalic cat have shown that both excitatory and inhibitory systems contribute to the locomotor drive potentials producing locomotor rhythm in motoneurones (Shefchyk & Jordan, 1985). It was shown that Renshaw cells, interneurones which act postsynaptically (directly onto motoneurones), are rhythmically active during locomotion and discharge in phase with the motoneurones from which they are excited (McCrea, Pratt & Jordan, 1980). Details of these findings were later presented by Pratt and Jordan (1987), specifying that flexor and extensor Renshaw cells became active after the onset of flexor and extensor motoneuronal activity, respectively.

Maximal activity in extensor Renshaw cells occurred at the end of the extension phase coincidental with the onset of hyperpolarization and a decrease in activity in extensor motoneurones. Maximal flexor Renshaw cell activity occurred during the middle to late flexion and was temporally related to the onset of reduced flexor motoneurone activity.

Nevertheless, postsynaptic inhibition onto α-motoneurone would not only decrease the reflex response, but also the EMG activity. These conclusions were drawn following a mathematical analysis of a model of the motoneuronal pool (Capaday & Stein, 1987b), accompanied with experimental testing of the results in decerebrate cats (Capaday & Stein, 1989). Although it might be possible that an additional source of excitation may interfere in order to maintain a constant level in the EMG activity, both mathematical analysis and computer simulations indicated that adding sufficient excitation would also restore the reflex response and not show any differences in the slope of the relationship between H-reflex amplitude and background muscle activity when comparing standing with walking and running tasks (Capaday & Stein, 1987b).

Several studies have also shown that the H response in the soleus muscle was modulated within the walking cycle in humans (Capaday & Stein, 1986, 1987a, Crenna & Frigo, 1987; Yang & Whelan, 1993) and in decerebrate cats (Akazawa *et al.* 1982). In all these studies, the amplitude of the H-reflex varied in parallel with the level of activity in the soleus muscle, suggesting that the variation may be caused by a change in the threshold of motoneuronal excitability. The concept for which the size of the reflex matches the amount of activity in the muscle has already been proposed by Matthews (1986) and is referred to as "automatic gain control".

Support for variations in motoneuronal excitability can be found in animal studies where the nerve activity is recorded during fictive locomotion. Alternating appearance of bursts of discharge in the nerves to hindlimb flexors and extensors in paralyzed thalamic cats have demonstrated that locomotor rhythmicity exists even in the absence of peripheral phasic afferent inflow (Bayev, 1978a). In fact, the pattern of efferent activity during fictive locomotion has been extensively studied in the decorticate cat (Perret & Cabelguen, 1976, 1980) and can be considered to follow the general description for phasic EMG activity recorded in the intact or deafferented limb. Hence, activation of different hindlimb muscles during stepping is realized according to a central locomotor program. It is therefore possible that the suppression of the H-reflex during the swing phase is simply a result of hyperpolarization of the soleus motoneurone pool. However, Yang and Whelan (1993) did not favour this possibility when they showed that in human subjects trained to activate their soleus muscle during the swing phase of the step cycle (when it is normally silent), the amplitude of the H-reflex remained attenuated.

Finally, another factor which could suppress the amplitude of the soleus H-reflex postsynaptically in the swing phase of the step cycle is the activation of its antagonist, the tibialis anterior muscle. It was shown that, under static conditions, an activation of the tibialis anterior led to a strong inhibition of the soleus H-reflex (Gottlieb, Agarwal & Stark, 1970; Morin & Pierrot-Deseilligny, 1977; Kagamihara & Tanaka, 1985; Crone & Nielsen, 1989). Also, the depression in the H-reflex observed in the swing phase was inversely correlated with the amplitude of the tibialis anterior muscle activity (Edamura, Yang & Stein, 1991). However, this hypothesis was later refuted since the depression in the H-reflex remained,

even in human subjects who could voluntarily not activate their tibialis anterior muscle during the step cycle, including during the swing phase (Yang & Whelan, 1993).

1.3.2 Presynaptic inhibition

Evidence presented in the preceding section suggest that the neural mechanisms which are responsible for the attenuation of reflexes during locomotion , as well as for the modulation of the reflex within the step cycle, may be acting upon the presynaptic terminal, rather than postsynaptically. Studies looking at presynaptic inhibition have been performed on animals with direct recording from presynaptic and postsynaptic neurones, and in humans with various indirect methods.

The classic technique for demonstrating presynaptic inhibition in animal experiments was to show that the voltage and the conductance of the postsynaptic neurone were not modified at the same time as the size of a synaptic potential was reduced (Baldissera, Hultborn & Illert, 1981; Rudomin, Quevedo & Equibar, 1993). Thus, the inhibition was not generated on the postsynaptic cell, but was acting presynaptically on the release of transmitter by the afferent neurone. A large number of studies on animals were performed using either the Wall's method for excitability testing (Wall, 1958), or dorsal root potential, or intra-axonal recordings of afferents. Data from these studies indicate that phasic presynaptic mechanisms are activated during fictive locomotion in decerebrate, decorticate and spinal cats injected with L-DOPA (Bayev, 1978b, 1980, 1981; Bayev & Kostyuk, 1981; Dubuc, Cabelguen & Rossignol, 1985, 1988; Duenas & Rudomin, 1988; Gossard, Cabelguen & Rossignol, 1989; Gossard & Rossignol, 1990) as well as in nonparalyzed walking preparations (Dubuc, Cabelguen & Rossignol, 1985; Duenas *et al.* 1990).

These studies suggested that the spontaneous changes of polarization of primary afferent terminals accompanying locomotion could alter the afferent transmission in different phases of the locomotor cycle. A possible interpretation of rhythmic afferent membrane depolarization relies on a concept called primary afferent depolarization (PAD). The pattern of rhythmic membrane potential fluctuations generally observed in extensor motoneurones suggests that the presynaptic inhibition is increased during the flexion phase and relatively decreased during the extension phase, allowing a modulation of the synaptic transmission in most of group I, and possibly group II, muscle afferent pathways (Duenas & Rudomin, 1988; Gossard *et al.* 1991).

Rhythmic depolarization during locomotion has not yet been shown to cause a phasic inhibition of sensory transmission (Shefchyk, Stein & Jordan, 1984). In fact, phasic antidromic discharges, resulting from PAD and which may have an inhibitory effect by colliding with orthodromic spikes, were seen in flexor afferents but not in extensor afferents of the cat hindlimb (Gossard *et al.* 1991). Nevertheless, Duenas *et al.* (1990) demonstrated that dorsal root reflexes from triceps surae muscle afferents were always smaller during locomotion than at rest and were smallest during the flexion phase. These remaining uncertainties about the role of PAD on the synaptic transmission in reflex pathways suggest that presynaptic inhibition mechanisms may be more important in attenuating the monosynaptic reflex during locomotion when compared to other tasks, than in being the main generator of the modulation observed within a step cycle.

Although studies looking at the presence of presynaptic inhibition in humans have not been as popular as for the animals (for technical difficulties and ethical reasons), three methods could be or have been used: (1) dorsal root reflexes, (2) Hoffmann and tendon

reflexes, and (3) vibration (Morin, Katz, Mazieres & Pierrot-Deseilligny, 1982; Capaday & Stein, 1986, 1987a; Hultborn, Meunier, Morin & Pierrot-Deseilligny, 1987; for review see Stein, 1995). The H-reflex technique was the most commonly used method and the interpretation of the results from these studies has already been discussed above (section 1.3.1). With the use of vibration a marked inhibition of the H-reflex was observed, inhibition which is usually believed to be of presynaptic nature (DeGail, Lance & Neilson, 1966; Delwaide, 1973).

However, discrepancies in the results obtained between studies with paraplegics (Calancie, Broton, Klose, Traad, Difini & Ayyar, 1993; Faist, Mazevet, Dietz & Pierrot-Deseilligny, 1994) or with hemiplegics (Milanov, 1992; Faist *et al.* 1994) with the vibration technique, highlight the great difficulty of identifying the reflex changes which are solely due to presynaptic inhibition in human studies. Nonetheless, there is a substantial body of evidence which supports the hypothesis that presynaptic inhibition mechanisms are involved in attenuating the monosynaptic reflex during locomotion (Stein, 1995).

1.3.3 Muscle spindle sensitivity and fusimotor drive

Extensive investigations of muscle spindle morphology, starting with the detailed drawings of Ruffini (1898), have demonstrated the intricate structure of this receptor (Barker, 1966; Ovalle & Smith, 1972; Boyd, 1986). Meanwhile, parallel studies of the receptor's physiological properties progressed with the first recordings of muscle spindle afferents by Matthews (1933). Several of these studies led to an appreciation of the functional complexity of the muscle spindle, including: (1) the establishment of γ-motoneurones as a specific fusimotor system (Leksell, 1945; Hunt & Kuffler, 1951a), (2) the functional classification of

primary (Ia) and secondary (II) afferents (Cooper, 1961), (3) the subdivision of static and dynamic γ-motoneurones (Matthews, 1962), and (4) the quantitative analysis of the response characteristics of muscle spindle afferents (Matthews & Stein, 1969a,b; Poppele & Bowman, 1970; Hulliger, Matthews & Noth, 1977a,b). In the last 25 years, there has been a discernible shift in muscle spindle research towards investigations of the functional role for muscle spindles and fusimotor drive in motor control.

The response of Ia and II afferents to a variety of precisely controlled length changes, over a wide range of amplitudes and frequencies, has been studied over the years (Harvey & Matthews, 1961; Brown, Engberg & Matthews, 1967; Lennerstrand, 1968; Matthews & Stein, 1969a; Kröller, Grüsser & Weiss, 1985). The sinusoidal stretch has been one form of input widely used by those studying muscle spindle properties. By applying the sinusoidal stretch over many cycles the response of the afferents to length and velocity components of the length change could be averaged. Three variables could be measured using this technique: mean discharge level, the amplitude of the discharge rate from the mean level, and the phase advance of the afferent response in relation to the length change (Matthews & Stein, 1969a; Poppele & Bowman, 1970).

It was with the application of various amplitudes of sinusoidal stretch and measuring the averaged change in modulation of the spindle response that striking nonlinearities of the Ia response became evident. The change in Ia afferent discharge rate for a given length change was shown to decrease dramatically when the amplitude of the stretch exceeded 0.1% of total muscle length (Matthews & Stein, 1969a). Functionally, this nonlinearity allows the Ia afferents to be very sensitive to small deviations in muscle length preventing a saturation in the signal during larger length changes. In contrast, group II afferents responded with a

constant and relatively low sensitivity (modulation amplitude in imp/s per mm of stretch) to all amplitudes of stretch.

The activity of muscle spindle afferents is determined by the complex interactions of two inputs: muscle length and γ-motoneurone activity (Hulliger, 1984). In general, stimulation of static γ-motoneurones enhances the steady-state discharge from the primary afferents during the static phase of stretch, reducing its dynamic responsiveness (Crowe & Matthews, 1964; Taylor, Stein & Murphy, 1985). On the other hand, stimulation of dynamic γ-efferents enhances the high-frequency burst of the Ia afferent signal during the dynamic phase of stretch. An understanding of the sources of γ-motoneurone recruitment is therefore imperative for the complete elucidation of muscle spindle function. At the peripheral level, γ-motoneurones receive input from skin, joint and muscle afferents (Murphy, 1978, Murphy, 1981; Ellaway, Murphy & Tripathi, 1982; Johansson & Sojka, 1985; Johansson, Sjölander & Sojka, 1986; Appelberg, Johansson & Sojka, 1986). The effects of these peripheral receptors on γ-motoneurone activity are quite variable and widespread. Also, the strength of these projections seems to depend on the experimental preparation as well as on the task performed by the animal.

Considering the large amount of efferent and afferent projections involved, it is not surprising that several roles have been proposed for the muscle spindle and γ-motoneurones. Merton (1953) led the way with his proposition of the follow-up servo hypothesis. The basic premise of his theory states that for all but the fastest movements, muscle activation is driven through a segmental reflex arc. This means that a command signal from the supraspinal centers first recruits the γ-motoneurones, resulting in an excitation of spindle afferents, which

in turn monosynaptically excites the α-motoneurones to produce the intended movement. However, Vallbo (1973) recorded from human nerve fibres with fine tungsten electrodes and clearly demonstrated that the muscle activation preceded the spindle afferent discharge. Furthermore, it was apparent that the rhythmical activation of α-motoneurones during respiration and locomotion was possible in a deafferented preparation (section 1.2).

A related concept known as the 'servo-assistance' theory was proposed and stated that the command signal which is responsible for generating a movement is delivered to both γ- and α-motoneurones, simultaneously. In the presence of unexpected loads or a rapid decrease in force production by the muscle, the discharge of the γ-motoneurones would provide the appropriate excitation to the spindle afferents in order to increase the activity of α-motoneurones (Matthews, 1981).

However, from recordings of muscle spindle receptor discharge activity in freely moving cats, it was inferred that, during natural movements, there is more to γ control than an invariant linkage of α and γ activation (Prochazka, Westerman & Ziccone, 1976,1977; Prochazka, Stephens & Wand, 1979; Loeb & Duysens, 1979). The results suggested that the amount and type of γ activation (static or dynamic) is preset at a fairly steady level, which varies according to the specific task or context. This hypothesis was later verified with simulations performed during acute experiments on anaesthetized cats (Hulliger & Prochazka, 1983; Hulliger, Zangger, Prochazka & Appenteng, 1985) and the term 'fusimotor set' was proposed for describing the balance of fusimotor action to a muscle at any given time (Prochazka, Hulliger, Zangger & Appenteng, 1985).

Results from the various behavioural tasks are summarized in Prochazka, Hulliger, Trend and Dürmüller (1988). Generally, both static and dynamic γ-motoneurones are set at higher levels as the speed and difficulty of the movement increase. Unpredictable conditions, as when a cat is picked up or handled, lead to marked increases in dynamic fusimotor activity reflected in greatly increased spindle responsiveness. When the animal is performing a difficult task, such as walking across a narrow beam, high levels of both static and dynamic γ-drive are present. Thus, by adjusting the balance between activation of static and dynamic gamma motor neurones, the nervous system uses the fusimotor system to fine-tune the spindles, so that the ensemble output of the muscle spindles provides the most appropriate information for the specific task.

Notwithstanding such variation in the 'fusimotor set', a generalization has emerged for rhythmic movements such as locomotion, respiration and mastication. It is proposed that extrafusal muscle contractions are accompanied by coactivity in static and dynamic γ-motoneurones (Murphy & Martin, 1993), thus optimizing spindle afferent feedback for reflex contributions to muscle contraction (section 1.4.1 below). Moreover, afferent recordings in humans have generally failed to show convincing fusimotor set in absence of α activity (Gandevia & Burke, 1985; Ribot, Roll & Vedel, 1986). It is noteworthy to mention that single fibre recordings in humans have only been achieved by severely restricting the range of motor tasks.

In experiments, in which direct recordings from static and dynamic γ-efferents were performed in anaesthetized or decerebrate cats during locomotion (Murphy, Stein & Taylor, 1984; Bessou, Cabelguen, Joffroy, Montoya & Pagès, 1986; Murphy &

Hammond, 1993), both tonic and phasic (α-linked) patterns of γ discharge have been encountered. For example, during stepping, fusimotor activity was recorded from dissected filaments of the nerve to triceps surae muscles in the decerebrate cat. Some neurones showed little modulation and fired throughout the walking sequence, while other neurones were deeply modulated, in parallel with α activity, with each step (Murphy *et al.* 1984). These patterns were found to correspond to static and dynamic γ efferents, respectively.

Control experiments confirmed the general role of fusimotor drive on the sensitivity of muscle spindle afferents: stimulation of dynamic γ-motoneurones increased the sensitivity to sinusoidal stretch while stimulation of static γ-motoneurones decreased it (Taylor *et al.* 1985). These results are in accordance with the firing behaviour of soleus muscle spindle afferents recorded in premammillary cats where there was a decrease in the spindle sensitivity to stretch during walking, despite the large increase in firing rates (Taylor *et al.* 1985). This switch in fusimotor drive might be responsible for the reduction in the stretch reflex response seen during walking in the mesencephalic cat (Akazawa *et al.* 1982). Nonetheless, it does not account for the modulation seen throughout the step cycle since the H-reflex also exhibited a phase-dependent modulation (Akazawa *et al.* 1982). A direct stimulation of the group I afferents (as used in the H-reflex method) bypasses the muscle receptors, and therefore, the electromyographic reflex responses are not affected by changes in the spindle sensitivity.

Such a reduced muscle spindle sensitivity in ongoing movements was also observed in recordings from intact cats (Prochazka *et al.* 1985; Hulliger, Dürmüller, Prochazka & Trend, 1989). Until now, few studies have looked at muscle spindle firing in the spinalized

animal (Sjöström & Zangger, 1975, 1976). The results from these few studies displayed an increase in γ-drive during the locomotor rhythm, and this γ input was inferred to be of static origin only in the ankle flexor muscles. Thus, it remains uncertain whether the increase in static γ-drive observed in ankle extensors of intact and decerebrate cats is still present after spinalization, thus arising from the locomotor circuitry located in the spinal cord. This issue is the object of the second study presented in this thesis where the activity in soleus muscle spindle afferents was recorded in spinalized cats during Clonidine- or DOPA-induced locomotor rhythm (Chapter 3).

1.3.4 Gating of interneuronal pathways

Spinal interneurones are neurones that are restricted to the spinal cord and which influence other nearby neurones. Interneurones form spinal networks that mediate between input and output elements and produce varied reflex behaviours. Divergence and convergence are two basic mechanisms of integration used by the interneurones in order to relay information from sensory to motor neurones. Divergence refers to the distribution of the output of a single neurone to a number of target neurones and is present in virtually all spinal neurones. For example, Ia afferent fibres from the muscle spindle branch extensively in the spinal cord, terminating on most homonymous motoneurones, on many synergist motoneurones and onto interneurones that inhibit antagonist motoneurones. Convergence refers to the processing of input from several neurones onto one neurone. All motoneurones and interneurones receive convergent input and thus their activity at any given time reflects the summation of excitatory and inhibitory PSPs from several sources.

As mentioned earlier, a modulation in reflex response was observed when a cutaneous stimulus was applied at different times during the walking cycle. Low threshold cutaneous stimulation during swing increased flexor EMG amplitude and duration, as well as the overall cycle duration. The same stimulation during stance increased extensor EMG amplitude and also increased the cycle duration. This reflex behaviour occurred in intact, decerebrate and spinalized cats (for references see section 1.1). A similar phase-dependent modulation of cutaneous reflexes was also observed in man (Crenna & Frigo, 1984; Bélanger & Patla, 1987; Duysens *et al.* 1991). Moreover, a reversal in the sign of the cutaneous reflex was observed in the human tibialis anterior muscle during locomotion (Yang & Stein, 1990; Duysens *et al.* 1990).

These reflex pathways involve one or more sets of interneurones interposed between afferent input and motor output. Excitation or inhibition of the spinal interneurones by signals from supraspinal centers, central pattern generating circuitry and/or other segmental reflex pathways can enhance or suppress specific reflex actions in order to suit the particular behavioural context in which they occur. Sensory interneurones (which are directly excited by afferents) and premotor interneurones (synapsing directly onto the motoneurones) may be subject to postsynaptic modulation and thus, be involved in regulating the flow of sensory information. In fact, several lines of indirect evidence have already suggested that the convergence of sensory excitation and rhythmic premotor drive at interneuronal levels in the spinal cord is critically involved in sensory gating during locomotion (Sillar, 1991).

Investigating the role of interneurones in reflex pathways in mammals has proved difficult and very few categories of interneuron with a defined role in locomotion have been identified (for review see Jordan, 1983). Most valuable findings have come from simpler

vertebrates, such as the lamprey (Grillner & Dubuc, 1985) and the *Xenopus* embryo (Clarke & Roberts, 1984; Dale, 1985; Roberts, Soffe & Dale, 1986; Sillar & Roberts, 1988). For example, in the immobilized *Xenopus* embryo, sensory inputs from skin receptors excite a specific class of spinal sensory interneurones whose activity leads to reflex bending of the body away from the stimulus. During swimming, these inputs are gated by rhythmic postsynaptic inhibition, so that sensory drive reaches motor neurones only at phases in the locomotor cycle when the resulting contraction will likewise turn the embryo away from the stimulated side (Sillar & Roberts, 1988).

Similarly, during locust flight, the alternating inhibitory and excitatory synaptic drive that underlies rhythmic firing of motor and premotor neurones has been shown to modulate sensory feedback (Reichert & Rowell, 1985). Indeed, in the locust, descending information signalling deviation from the flight path is transmitted via two neural pathways: a direct pathway to the flight motoneurones, and an indirect pathway which involves thoracic interneurones. The direct pathway evokes short-latency PSPs which are independent of the behavioural state of the animal while, on the other hand, the interneurones from the indirect pathway are strongly influenced by the central rhythm generator. The data of Reichert and Rowell (1985) showed that the indirect pathway was more powerful in its effects on motoneurones than the direct pathway. The transmission of this particular sensory input was therefore more efficient during appropriate phases of the flight cycle.

Studies regarding cutaneous reflex pathways were also conducted in spinalized and paralyzed cats at rest (Schomburg & Steffens, 1986) and during locomotion (Andersson *et al.* 1978; Schomburg & Behrends, 1978; Forssberg, 1981; Schomburg, Behrends & Steffens, 1981; Schmidt, Meyers, Fleshman, Tokuriki & Burke, 1988; Schmidt, Meyers,

Tokuriki & Burke, 1989; LaBella, Niechaj & Rossignol, 1992). At rest, the responses to a noxious stimulus (radiant heat) in the triceps surae motoneurones displayed a rather complex pattern. The depolarizations were small and variable, suggesting the presence of two distinct pathways: an excitatory one and an inhibitory one, which together produced the mixture of excitatory and inhibitory PSPs observed in the extensor motoneurones (Schomburg & Steffens, 1986). These parallel interneuronal pathways are believed to be responsible for the phase-dependent reflex responses observed during locomotion (Schomburg & Behrends, 1978; Schomburg *et al.* 1981; Schmidt *et al.* 1989). Effectively, a facilitation of the excitatory pathway and a depression of the inhibitory pathway to the active motoneuronal pool, with a concomitant facilitation of the inhibitory pathway and depression of the excitatory pathway to the antagonistic motoneurones, are likely to generate the phase-dependent reflex response seen during locomotion.

Furthermore, the results from Andersson *et al.* (1978) indicated that there are several pathways involved in the responses of different latency ranges in the same or different muscle groups. These responses are differentially modulated suggesting that each pathway transmits maximally in one phase and minimally in another phase. The finding that the response amplitudes in the different latency ranges were not all modulated in the same phase strongly suggest that the amplitude of the responses is not a function of the motoneuronal excitability, but rather of premotoneuronal elements. Since these results were obtained in high spinal and paralyzed cats (therefore in the absence of descending and peripheral afferent inputs), it appears that the central rhythm generator would be involved in appropriately gating these interneuronal pathways (Schomburg & Steffens, 1986).

The existence of these parallel interneuronal pathways constitutes the support for one of the hypotheses put forward in order to identify the neural mechanism responsible for the phase-dependent reflex reversal seen in the human tibialis anterior muscle during walking (Chapter 2). The other two hypotheses rely on the presence of a task-dependent distribution of motoneurones supplying the muscle. It was proposed that a selective activation of a specific subpopulation of motor units at each phase of the step cycle may account for the modulation observed in the phase-dependent reflex response (Hoffer, Loeb, Sugano, Marks, O'Donovan & Pratt, 1987; Chanaud, Pratt & Loeb, 1991; Gordon, Loeb & Richmond, 1991).

1.4 ROLE OF REFLEXES DURING LOCOMOTION

Although it is now well accepted that sensory feedback has a role to play in regulating rhythmic movement, particularly locomotion, the mechanisms involved in each sensory pathway are still under investigation. The stretch reflex is believed to be responsible for producing part of the ankle extensor activity during the stance phase of a step cycle and a summary of the studies regarding this issue is given below. Also, the roles for hip extensor receptors as well as group Ib afferents are briefly reviewed.

1.4.1 Stretch reflex contribution to locomotor pattern

Over 25 years ago, Severin (1970) reported that in the early stages of a peripheral nerve block, one can selectively block the fusimotor axons. During this time period the

size of the EMG burst of the triceps surae muscles, normally seen during the extension phase of the walking cycle, was reduced by 50%, implying that muscle spindle activity contributes substantially to the activation of this muscle group. The fact that ankle extensors undergo an active lengthening contraction through most of the stance phase in both cats and humans, when added to the results from Prochazka *et al.* (1976) showing that muscle spindle afferents fire during this lengthening contraction, indicated that the stretch reflex likely contributes to the EMG activity. Moreover, H-reflex studies in cats (Akazawa *et al.* 1982) and in humans (Capaday & Stein, 1986; Crenna & Frigo, 1987; Dietz *et al.* 1990), have shown that transmission through group Ia monosynaptic pathways is maximal during the stance (extension) phase, suggesting that the active muscle lengthening will most likely provoke an excitatory reflex response.

Small, rapid stretches have been applied to the ankle extensor muscles during the stance phase of walking by lifting the forefoot with a pneumatic device (Yang *et al.* 1991). The results suggested that a velocity sensitive element contributes substantially to the activation of the soleus muscle through the stance phase, when the muscle is actively stretched. The contribution of the primary spindle feedback was estimated between 30% and 60% of the early soleus activity, most likely caused by velocity-sensitive peripheral afferents. Note that the earlier estimate of 50%, proposed by Severin (1970), falls within this range.

In another study using a mechanical perturbation of the human ankle, it was estimated that up to 50% of the activity present in the soleus muscle was of reflex origin, at the early stage of the stance phase (T. Sinkjær, personal communication). This result is in contrast with the findings of Stein and Kearney (1995), obtained from mechanical

perturbations applied to the human ankle joint. By superimposing pulse displacements to random perturbations, Stein and Kearney demonstrated that the reflex responses (to the pulse inputs) decreased in a graded manner with increasing amplitude or bandwidth of the random perturbations. This led them to suggest that, from the time of heel contact until the foot is flat on the ground (early part of the stance phase), the stretch reflex would be substantially inhibited by afferent activity prompted by heel strike and initial shortening. This could be functionally significant since at that time, reflexes are counterproductive, slowing the forward momentum of the body (Stein & Capaday, 1988). On the other hand, reflexes are useful during the second part of the stance phase, propelling the body up and forward, and this is the time when they were shown to contribute to the force generation (Yang *et al.* 1991).

These discrepancies clearly demonstrate that the stretch reflex contribution to the muscle activity during the stance phase has yet to be determined accurately. Furthermore, the shape of the applied perturbation seems to have a prominent influence on the estimated value. This question was addressed in the current thesis by estimating the gain of the stretch reflex loop from data recorded with sinusoidal perturbations applied to the triceps surae muscles in decerebrate and spinal cats (Chapter 4).

1.4.2 Other afferent contribution to locomotor pattern

Afferents from muscle spindles and Golgi tendon organs convey different information: changes in muscle length for the muscle spindle afferents and variations in the muscle tension for the Ib afferents (innervating the Golgi tendon organs). Recordings from these afferents were first done by Matthews (1933). The results obtained then suggested that

the muscle spindle afferents made excitatory connections to homonymous muscles while the afferents from the Golgi tendon organs were acting via an inhibitory pathway (both with a reverse influence onto antagonistic muscles). Until then, the Ib inhibition was regarded mainly as a protective reflex against muscle overloading, because of the high threshold of the receptors to passive stretch. This view was already challenged with the demonstration that active contraction of only a few motor units (or even a single unit) may activate tendon organs (Matthews, 1933; Hunt & Kuffler, 1951b; see review by Haase, Cleveland & Ross, 1975; Baldissera *et al.* 1981). Homonymous tendon organ inhibition provides a negative feedback mechanism for regulating muscle tension, such as the negative feedback from muscle spindles which regulates muscle length. Thus the Ib afferent system would tend to counteract small changes in muscle tension by increasing or decreasing the inhibition to the motor neurones. However, evidence obtained within the last ten years are now establishing that group Ib afferents switch from having an inhibitory influence to providing an excitatory input onto homonymous motoneurones at the onset of locomotion in the ankle extensor muscles (Conway, Hultborn & Kiehn, 1987; Pearson, Ramirez & Jiang, 1992; Pearson & Collins, 1993; Gossard, Brownstone, Barajon & Hultborn, 1994; Guertin, Angel, Perrault & McCrea, 1995; Whelan, Hiebert & Pearson, 1995; McCrea, Shefchyk, Stephens & Pearson, 1995).

Two functions have been proposed for this excitatory action of extensor Ib afferents onto the extensor motoneurones during walking: (1) regulation of the transition from stance to swing by ensuring that the swing phase is not initiated until a limb is unloaded (Duysens & Pearson, 1980; Conway *et al.* 1987), and (2) reinforcement of ongoing activity in leg extensor muscles during stance that would provide a mechanism for automatic load

compensation (Pearson & Collins, 1993; Guertin *et al.* 1995; Whelan *et al.* 1995; McCrea *et al.* 1995). This autogenetic excitation, present during the stance phase, would add to the monosynaptic excitation from group Ia afferents and contribute to part of the EMG activity of the extensors, as discussed above (section 1.4.1).

Afferents responding to the hip position also play an important role in the control of locomotion. They sense the position of the limb in the step cycle and signal when the limb has reached a posterior position in the stance phase in which the limb should switch to the swing phase (Grillner & Rossignol, 1978b). Moreover, this sensory input is so powerful that it was seen to entrain the CPG (Andersson & Grillner, 1981, 1983; Kriellaars, Brownstone, Noga & Jordan, 1994; Hiebert, Whelan, Prochazka & Pearson, 1996).

These two sensory mechanisms for the regulation of locomotion will not be discussed any further. More information can be found in a recent review by Pearson (1995).

1.5 REFLEX LOOP MODELLING

Most of the studies concerning the role of sensory feedback in the generation of rhythmic movements have dealt with the issue of whether or not a particular feature of the output pattern depends essentially on the presence of some afferent system. This approach has sometimes overshadowed a, perhaps more important point, which is that the output of the system in open-loop situations does not predict the output in closed-

loop conditions. Indeed, looking at a system with and without afferent feedback is really looking at a system operating in two radically different modes. In the open-loop mode, the system is internally calibrated from data that have been incorporated in the neural elements as a result of evolution and learning. In the closed-loop mode, the system is continuously recalibrated (adapted) by feedback so that it can work with more accuracy, more stability, and with a greater range of frequencies (bandwidth). It is thus adapted to take into account the nonlinearities of the motor system and the continuously changing conditions in which the movements have to be executed.

System analysis techniques have been used for up to four decades now in the study of the neuromuscular system and specifically in the study of the postural loop. These techniques allow for a more global view of the reflex systems, although most of the time, the models which are developed are oversimplified and incomplete. A brief review of the insights on the stretch reflex loop, provided by modelling studies, is given below.

1.5.1 Early models of the stretch reflex loop

Attempts of quantifying separately each of the main components of the stretch reflex loop are reported in the literature (see Agarwal & Gottlieb, 1982, 1984, 1985, for extensive reviews). For ethical reasons, these studies were mostly performed on animals. However, many investigators preferred to attack the problem by looking at the input/output relationship of the whole loop, especially for human studies. Both approaches have provided some significant insights into the mechanisms of postural control, even though no model that can perfectly represent the stretch reflex loop has been proposed yet.

Modelling of the complete stretch reflex loop was first attempted around the middle of the century (Adolph, 1959). Adolph considered an isometric condition at the knee joint while the quadriceps (extensors) and semitendinosus (flexors) muscles were exerting a constant force. He assumed that the muscle spindles had very little contribution as compared to the feedback due to tension changes in the isometric condition. Consequently, he proposed a model which was based on the response of the Golgi tendon organs to step changes, but he had no experimental results to support his model.

Meanwhile, Hammond (1960) presented a model with which he attempted to explain that the two EMG responses to stretch which occur at different latencies (early and late responses) could have their origin in two separate sensory endings. He suggested that the muscle servo responds as if there were: (1) detectors of acceleration or higher derivatives within the muscle having a short reflex arc and high sensitivity, (2) detectors of velocity within the muscle with a longer latency feedback path, and (3) detectors of position either in the muscle or outside it with a low sensitivity. Unfortunately, when he tried to integrate these equations into a closed-loop model, he discovered that his system would become highly unstable and concluded that the measured characteristics of the loop were not totally valid for describing the steady-state conditions, perhaps because they were obtained with transient stimuli. This problem of steady-state versus transient analysis of the closed-loop system has not changed much since this pioneering work.

1.5.2 Modelling of the stretch reflex loop with animal studies

Terzuolo and his colleagues made extensive measurements of various components of the stretch reflex loop in decerebrate cats, and discussed the influence of each of these

subsystems on the behaviour of the stretch reflex when integrated into a model. Poppele and Terzuolo (1968) studied the dynamic behaviour of the following subsystems with stretch inputs applied to the cat triceps surae muscles: (1) primary ending firing mechanism, (2) synaptic processing (transmembrane potential of motoneurone), (3) encoding process (motoneurone firing train), and (4) muscle mechanics effect. They looked at the overall myotatic loop by measuring the resulting active tension produced as the system output. One of their main results was that the stretch reflex had an approximately flat gain and near zero phase with respect to the input displacement over the range of input frequencies (0.2-6 Hz) and amplitudes (up to 2.5 mm) explored.

Rosenthal, McKean, Roberts and Terzuolo (1970) repeated this previous study for both the soleus and gastrocnemius muscles of a decerebrate cat. From looking at the relations between input displacement and output tension after sectioning the dorsal roots (open-loop conditions), they reported that changes in muscle tension resulting from the application of sinusoidal stretch inputs under closed-loop conditions were also sinusoidal, within the limits of the input frequencies and amplitudes tested. Also, the absolute gain of the loop was found to be related to the mean muscle tension. Moreover, it appeared that the dynamic characteristics of muscle and connective tissues (open-loop) were different from those of the stretch reflex. They suggested that this could be related to the recruitment process of the motoneurones in response to the stretch.

In addition, when tested under static fusimotor drive, the limits of the linear behaviour of the Ia afferents were greatly extended. This last result was further analyzed by Chen and Poppele (1978) in a study where they looked at the effect of fusimotor drive (either static or dynamic) on the cat muscle spindle responses to small and large amplitude sinusoidal

stretches. Without any γ-input, the Ia afferents were much more sensitive to small amplitude stretches than to larger one (see also Matthews & Stein, 1969a; Hulliger *et al.* 1977a). However, when the static γ-efferents were stimulated, the depth of modulation in the afferent firing rate was greatly reduced, and was rather constant for a larger range of stretch amplitudes, confirming the linearizing effect of static γ-drive on the Ia afferent firing behaviour, reported by Rosenthal *et al.* (1970).

In the meantime, Roberts, Rosenthal and Terzuolo (1971) assembled the data obtained in earlier studies and developed a linear control model for the stretch reflex loop. By fitting experimental data to simulated traces, they came to these four conclusions about the dynamic behaviour of the reflex loop: (1) the stability of the closed-loop system is very high, (2) the net phase shift around the reflex arc, in decerebrate cat, is quite modest, at least up to 8 Hz, (3) the presence of a limit-cycle oscillation such as tremor cannot be attributed to the dynamic characteristics of the subsystems responsible for the stretch reflex, and (4) the transport delay is not likely to play any significant role in the genesis of tremor.

This question of the origin of tremor was further investigated by Nichols, Stein and Bawa (1978) in the premammillary cat. A maintained tremor was observed and its dependence on the dynamics (elasticity or inertia) of the external load attached to the muscle was evaluated. In contrast to the conclusion stated above from Roberts *et al.* (1971), Nichols *et al.* (1978) presented some evidence to support their hypothesis that the observed tremor represents a feedback oscillation in a spinal pathway. First, the tremor could be reset by electrical stimulation of the muscle nerve. Second, the motoneurones fired in regular bursts which occurred at the frequency of oscillation, while the muscle receptors discharged a little less than 10 ms prior to the motoneurone discharge, even though the frequency of the tremor

varied over a wide range. Finally, the changes in the properties of the mechanical components of the oscillatory system (varying the mean tension of the muscle by changing length or level of activity, or by altering the mechanical properties of the external load) led to expected changes in the frequency and amplitude of tremor. These experimental results were in concordance with the theoretical approach proposed by Stein and Ogustöreli (1976) in their closed-loop model. They proposed that a high value of velocity sensitivity tends to produce oscillation which could be beneficial for linearizing muscle properties. In their analysis, the muscle, the load and the spindle were taken to be linear transfer functions with the spindle being sensitive to position, velocity and acceleration.

Meanwhile, Houk, Singer and Goldman (1970) designed a set of experiments to test whether the static gains of the reflex pathways were sufficiently large to provide the postural adjustments often ascribed to them in the presence of various disturbances. In decerebrate cats, they attempted to evaluate the length and force feedback to soleus muscles during two reflexive conditions: (1) mechanically applied stretch of the muscle, and (2) internal disturbing force applied via the stimulation of ventral root filaments. Following an estimation of some mechanical properties of the muscle, their results indicated that length feedback from spindles increases the stiffness of the stretch reflex fivefold over what it would be if the muscle were regulated without feedback. As for the force feedback, the effect is rather small. The gains of both spindle and tendon organ pathways increased approximately as the square root of the operating force. Therefore they proposed a model which might explain the variation seen in the reflex stiffness with an increase in the operating force. In a review paper about the regulation of stiffness by skeletomotor reflexes, Houk (1979) suggested that an adjustment of the gain of both length and force feedback loops would determine the skeletomotor output.

Based on results obtained from the above study performed with decerebrate cats and from a study on the regulatory actions of the stretch reflex at the human elbow joint, Crago, Houk and Hasan (1976) concluded that stiffness is the main variable controlled by the motor system and that a change in skeletomotor output is proportional to the difference between the regulated stiffness and the actual stiffness. This conclusion was also supported by experimental data, also obtained in decerebrate cats, where reflex nonlinearities were seen to compensate for the nonlinearities in the muscle properties (Nichols & Houk, 1976). However, differences in reflex amplitude, observed in mesencephalic cats when levels of muscle excitation were matched during posture and locomotion (Akazawa et al. 1982), suggested that muscle receptors were not simply functioning to compensate for muscle properties in the regulation of stiffness, as proposed by Nichols and Houk (1976).

1.5.3 Modelling of the stretch reflex loop with human studies

Gottlieb and Agarwal (1980) asked their subjects to produce a phasic voluntary flexion of the foot about the ankle while they applied a torque to perturb the movement and evoke reflex responses. The consequences of applying this perturbation in conjunction with a rapid phasic voluntary movement were seen in the change in the myotatic response, according to the time of occurrence of the perturbation relative to the onset of the movement. When the perturbation occurred at the onset or no more than 50 ms after the onset of the voluntary contraction, there was a dramatic increase in the myotatic component of the response. In contrast, when the perturbation occurred more than 50 ms after the onset of the voluntary EMG, the disturbing torque was far less effective in altering the movement of the foot (reduction in the evoked myotatic or postmyotatic response in the muscles that were

contracting). These results suggested the presence of a reflex gain modulation with the execution of voluntary movements. Recent studies, using random inputs as perturbations, highlighted the fact that, not only active contractions, but also passive movements can strongly modulate the magnitude of the stretch reflex (Kirsch, Kearney & MacNeil, 1993; Kirsch & Kearney, 1993; Stein & Kearney, 1995).

The issue of stiffness regulation, initially proposed by Nichols and Houk (1976), was recently studied in experiments on the human hand (Carter, Crago & Keith, 1990). The total stiffness of a metacarpal joint was measured and decomposed in three individual components: passive, intrinsic and reflex stiffnesses. The results indicated that the total stiffness is no better regulated as the initial torque is varied when the reflexes are intact than when the reflexes are absent. Rather, reflexes seem more likely to act towards yield compensation, and to linearize the inherently nonlinear mechanical properties of areflexive muscles.

Further evidence against the stiffness regulation hypothesis of Nichols and Houk (1976) were recently obtained from studies looking at the influence of the instruction given to the subjects on the stretch reflex behaviour (Doemges & Rack, 1992; Bennett, Gorassini & Prochazka, 1994). The relative roles of intrinsic stiffness, short-latency and long-latency reflexes, and voluntary reactions were assessed in a series of experiments that involved stimulation and voluntary activation of elbow flexor muscles in a variety of tasks including catching a heavy ball (Bennett et al. 1994). The results emphasized that the subject's intentional reactions (including the predictive and voluntary components of the response) were far more effective in maintaining the position of the hand after the ball was caught than the intrinsic and reflex stiffnesses alone. Therefore the extent to which the stretch reflex can augment the inherent muscle stiffness appeared to be limited.

Finally, a linear increase in the reflex responses in human elbow flexor muscles was observed over a large range of tonic forces (Stein, Hunter, Lafontaine & Jones, 1995), corroborating the concept of 'automatic gain control' proposed by Matthews (1986). This concept stipulates that, for a given change in length, the reflex force increases with the tonic level of force being exerted. This idea is in contrast to the stiffness regulation hypothesis presented by Nichols and Houk (1976).

The gain of the stretch reflex loop has been estimated in two different ways in the studies presented in the following chapters. First, experimental data acquired from a sinusoidal stretch of cat triceps surae muscles was used to calculate the gain of the reflex loop in a similar manner than for the study of Bennett et al. (1994; see Chapter 4). Second, a simplified model of the mammalian stretch reflex loop was utilized to simulate the experimental data obtained while stretching the cat triceps surae muscles with pulse displacements (Chapter 5). The gain of the reflex loop was then computed, using the parameters of the model providing the best fit with the experimental data. In both cases, the gain of the stretch reflex loop was evaluated for stretches applied during tonic contractions in the decerebrate cat, and during walking in the spinalized animal. Since there is a modulation in the stretch reflex response between tasks, as well as within the locomotor cycle (Akazawa et al. 1982), it was interesting to see if the loop gain reflects these variations.

1.6 SUMMARY OF OBJECTIVES

Rossignol *et al.* (1988) subdivided their review about the role of sensory inputs on the control of rhythmic movements according to the three methods most generally used to study how afferent feedback is involved in regulating patterns of rhythmical movements in higher vertebrates: (1) recording of the discharge of afferents during movement; (2) removal of the afferent feedback through either deafferentation or paralysis, and (3) selective stimulation of afferents. All three of these methods were used in the studies presented in this thesis. Indeed, stimulation of cutaneous afferents was used to identify the neural mechanism which causes a phase-dependent reflex reversal in the human tibialis anterior muscle during walking (Chapter 2). Then, recording of the discharge of soleus muscle spindle afferents during tonic and phasic contractions was performed on decerebrate (eventually spinalized) cats (Chapter 3). Finally, removal of the afferent feedback through deafferentation was used to study the stretch reflex behaviour with various types of stretch, also with decerebrate (eventually spinalized) cats, during tonic and phasic contractions (Chapters 4 and 5).

The specific objectives of the studies described in this thesis were:

(1) to identify the neural mechanism responsible for the phase-dependent cutaneous reflex reversal observed in the human tibialis anterior muscle during walking (Chapter 2);

(2) to examine the fusimotor control on the soleus muscle spindle afferents in decerebrate cats during tonic contractions and, after spinalization, during locomotion (Chapter 3);

(3) to compare the soleus primary afferent firing behaviour during spinal locomotion induced by either Clonidine or DOPA (Chapter 3);

(4) to evaluate the gain of the stretch reflex loop by sinusoidally stretching the triceps surae muscles of decerebrate cats during tonic and phasic contractions (Chapter 4);

(5) to estimate what fraction of the extensor force is produced by the activation of the stretch reflex in the walking cycle (Chapter 4);

(6) to verify if the phase-dependent modulation of the stretch reflex seen in humans and decerebrate cats subsists after spinalization (Chapter 5);

(7) to contrast the dynamic and static reflex-mediated stiffnesses of the triceps surae muscles of decerebrate cats during tonic contractions (Chapter 5);

(8) to quantify the gain of the stretch reflex loop with simulations of the responses to a pulse stretch, and compare the results to the reflex loop gains calculated with a sinusoidal stretch (Chapter 5);

(9) to study the effect of changes in the external load stiffness on the gain of the stretch reflex during tonic and phasic contractions (Chapter 5).

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CHAPTER 2

MECHANISM FOR REFLEX REVERSAL DURING WALKING IN HUMAN TIBIALIS ANTERIOR MUSCLE REVEALED BY SINGLE MOTOR UNIT RECORDING

2.1 INTRODUCTION

Stimulation of cutaneous afferents from the foot during walking can greatly alter the muscle activity of walking and indeed the locomotor rhythm itself (reviewed in Rossignol, Lund & Drew, 1988). This is perhaps not surprising, since the foot provides important information about the nature and stability of the support surface. The responses elicited by cutaneous stimuli are very dependent on the phase of the walking cycle in which stimuli are applied. This is true of many different preparations of the cat including those that are intact (Duysens & Stein, 1978; Forssberg, 1979; Duysens & Loeb, 1980; Duysens, Loeb & Weston, 1980; Wand, Prochazka & Sontag, 1980; Abraham, Marks & Loeb, 1985), decerebrate (Duysens & Pearson, 1976) or spinalized (Forssberg, Grillner & Rossignol, 1975, 1977; Andersson, Forssberg, Grillner & Lindquist, 1978).

In spinalized cats, an innocuous stimulus to the foot enhances flexor activity during the swing phase and enhances extensor activity during the stance phase. This reversal in the response has been called "phase-dependent reflex reversal" (Forssberg *et al.* 1975). Activation of flexors in the swing phase when an obstacle is encountered would allow the

animal to step over the obstacle. Activation of extensors in the stance phase when an obstacle is encountered would reinforce the supporting function of the stance phase and prevent the limb from collapsing. Some qualitative differences were seen between the different preparations (Forssberg, 1979; Wand *et al.* 1980), but in general the responses elicited are functionally appropriate in permitting the cat to maintain stability and progression in response to an unexpected sensory stimulus during walking (Forssberg *et al.* 1975, 1977, Forssberg, 1979).

In humans, phase-dependent modulation of cutaneous reflexes during locomotion has also been reported (Crenna & Frigo, 1984; Bélanger & Patla, 1987). Indeed, a phase-dependent reflex reversal was found, in which the sign of the reflex changed from excitation of muscles in one phase of walking to inhibition of the same muscles in another phase (Duysens, Trippel, Horstmann & Dietz, 1990; Yang & Stein, 1990). For example, the tibialis anterior (TA) muscle is active in two parts of the walking cycle in humans: (1) starting from late stance and extending through most of the swing phase (from here on referred to as the swing phase) to dorsiflex the ankle to clear the foot from the ground, and (2) at the transition from swing to stance to control the lowering of the foot after heel contact. A stimulus delivered to the posterior tibial nerve during the swing phase generated a facilitatory response at a medium latency in the TA muscle. In contrast, when the stimulus was applied during the transition from swing to stance, it generated a suppressive response at approximately the same latency. This type of reversal in the sign of the response has been reported for some muscle groups in intact cats (Drew & Rossignol, 1987), decerebrate cats (Miller, Ruit & Van der Meché, 1977) and, for a small number of motoneurones, paralysed cats that were

undergoing fictive locomotion (Schomburg & Behrends, 1978; Schomburg, Behrends & Steffens, 1981).

The reversal in the sign of the response, as previously described, suggests three possible organizations of the reflex pathways:

FUNCTIONAL TASK GROUPS. There could be different subpopulations of motor units in a muscle, each being recruited at different times in the step cycle (Fig. 2.1*A*). Thus, for the TA muscle, motoneurones that are activated in the swing phase may be different from those recruited in the transition phase from swing to stance. Cutaneous afferents may have excitatory projections to the motoneurones that are activated in the swing phase, and inhibitory projections to the motoneurones that are activated during the transition from swing to stance. In this way, the reflex reversal is automatically generated as a function of which population of motor units is active.

REFLEX GROUPS. The motoneurones in the TA muscle could consist of one functional group. Motoneurones would be recruited in an orderly manner according to size (Henneman, Somjen & Carpenter, 1965). However, the pattern of connectivity from cutaneous afferents could be different for different subpopulations of motoneurones, with some receiving excitatory connections and others receiving inhibitory connections (Fig. 2.1*B*). Reflex reversal would result from alternate gating of the two pathways at different times in the step cycle.

PARALLEL PATHWAYS. The reflex reversal could result from an alternate gating of two parallel interneuronal pathways from cutaneous afferents innervating the same motoneurone (Fig. 2.1*C*). One of these pathways is excitatory and is activated during the

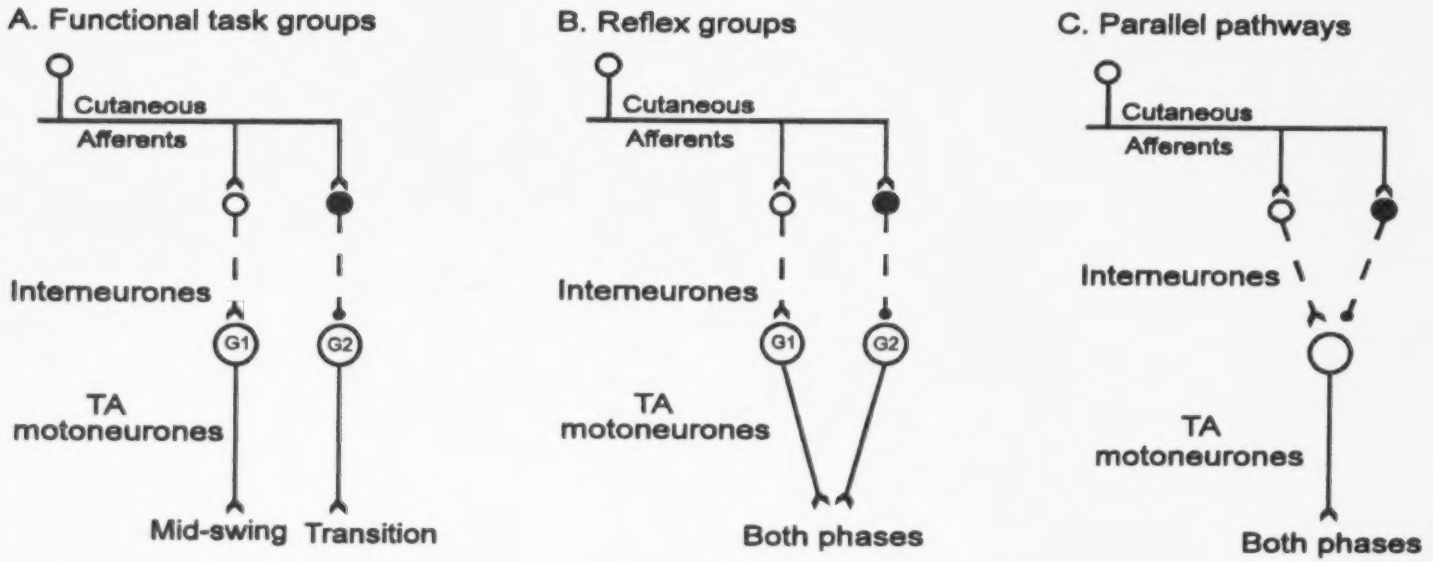

Figure 2.1 - Schematic diagram illustrating the hypotheses to be tested

A. Organization of the reflex pathways if functional task groups were the explanation for the reversal. The motoneurones from the tibialis anterior (TA) muscle would consist of two functional groups, one active in the swing phase (G1), and the other active at the transition from swing to stance (G2). Each group of motoneurones would have different connectivities with the cutaneous afferents: one pathway would result in excitation (open interneurone) and the other in inhibition (filled interneurone) of the motoneurone. There are an unknown number of interneurones between the primary afferent and the motoneurone, as indicated by the dashed lines on the interneurones. *B*. Organization of the reflex pathway if reflex groups accounted for the reversal. The motoneurones belong to one functional group, and are recruited as a function of their size during both phases of activity during walking. However, certain motoneurones are excited by cutaneous afferents (G1) while others are inhibited (G2) by the same afferents. Reflex reversal results from an alternate gating or dominance of one of the pathways. *C*. Organization of the reflex pathway if parallel pathways to single motoneurones explained the reversal. Motoneurones from the TA would all belong to one functional group (as in *B*). There would be excitatory and inhibitory pathways converging on each motoneurone from the cutaneous afferents. Reflex reversal would result from alternate gating or dominance of one of the pathways.

swing phase, while the other pathway is inhibitory and is activated during the transition from swing to stance.

In all three scenarios shown in Fig. 2.1, the projection pattern is shown for a single cutaneous afferent. It is also possible that different cutaneous afferents contained within the same nerve project to each of the pathways, a possibility that cannot be tested with the current experimental protocol. There is evidence from mammals to support all of these hypotheses (see DISCUSSION for details).

The goal of the present study is to distinguish between the three possible organizations of reflex pathways described above. This can be accomplished by recording from single motor units in the TA muscle during walking. If the reflex reversal results from the activation of two different subpopulations of the motoneurone pool (functional task groups), then different sets of TA motor units should be activated during each phase of the step cycle. If, however, the reflex reversal results from alternate gating of parallel pathways to the same motoneurone pool (reflex groups or parallel pathways), then most motor units should fire during both activity bursts of the TA muscle in the step cycle.

One can further distinguish whether these parallel pathways project to the same motor units (parallel pathways) or to different groups of motor units (reflex groups) by examining the responses of individual motor units to stimuli presented at the two different times in the step cycle. If a motor unit is excited by cutaneous stimuli applied during the swing phase of the walking cycle, and inhibited by stimuli applied during the transition from swing to stance, then the parallel pathways must both project to individual motoneurones.

As will be seen, the results support the hypothesis that there are parallel, excitatory and inhibitory pathways projecting to individual motoneurones. The different pathways are favoured at different times in the step cycle.

2.2 METHODS

2.2.1 Stimulation

Stimuli were applied over the posterior tibial nerve just behind the medial malleolus of the right leg with a surface electrode (Beckman type, 1 cm in diameter or Chattanooga, self-adhesive type, 1.5 cm in diameter). A large indifferent electrode was placed on the anterolateral surface of the ankle joint. The stimulus consisted of three consecutive monophasic pulses, 0.3 ms in duration, separated by 10 ms. Bipolar surface electrodes were placed on the abductor hallucis muscle for monitoring the compound muscle action potential (M wave), which indicated the stimulus strength. The stimulus intensity was selected to be between 1.5 and 1.7 times motor threshold for the abductor hallucis muscle (Yang & Stein, 1990). This intensity corresponds to about 2 times perceptual threshold, where perceptual threshold is defined as the intensity at which the subject first feels the stimulus radiating into the sole of the foot.

2.2.2 EMG recording from the TA muscle

Two sets of electrodes (surface and intramuscular) were used to record the electromyogram (EMG) of the right TA muscle. Bipolar surface electrodes (Beckman type,

1 cm in diameter) were placed vertically, 2 cm apart, about 5 cm distal to the tibial tubercle and 1 cm lateral to the anterior border of the tibia. The intramuscular electrodes consisted of three stainless-steel wires, 50 μm in recording diameter (California Fine Wire Company, Grover City, CA, USA), coated with insulating material. These wires were threaded through a 25 gauge needle to allow insertion into the muscle. The recording ends of the wires were glued together with medical grade silastic (Dow Corning). The wires were then bent close to the tip of the needle shaping a hook of a few millimetres long, and a cut was made perpendicular to the wires to form the recording surface. These intramuscular electrodes were gas sterilized prior to use. The wires were inserted near the origin of the muscle, about 1-2 cm proximal to the surface electrode. The needle was removed shortly after insertion, leaving the wires in the muscle for recording. The pair of wires that provided the best signal among three possible pairs was selected for recording. At the beginning of the session, the subjects walked for a period of about 5 min to allow the electrodes to stabilize within the muscle. A small blood clot often developed at the point of insertion of the wires, which seemed to help keep the electrodes in place. Both surface and intramuscular EMG signals were amplified and high-pass filtered at 10 Hz prior to recording. The leads from the intramuscular electrodes were kept separate from the other leads to minimize contact artifact.

2.2.3 Protocol

Twenty-six subjects participated in the experiments and gave their informed written consent before the start of the recording session. The experiments were approved by the Faculty of Rehabilitation Medicine Ethics Committee. The subjects walked on a treadmill at a low speed, which was adjusted to a comfortable pace for each individual (about 0.6 m/s).

An ankle splint (similar to an ankle-foot orthosis) was made of Sansplint material (Smith & Nephew, Lachine, Qué., Canada) for each subject. The splint was used to reduce motion at the ankle joint during walking, thus reducing the excursion of the TA muscle, to allow for more stable recordings of single motor units. Two footswitches were used, one underneath the heel and another one underneath the ball of the foot for recording the time of heel contact and toe lift, respectively.

In the first part of the experiment, the stimuli were delivered randomly in time throughout the step cycle, at intervals ranging from 1.5 to 4 s. No more than one stimulus was delivered per step. The surface EMG signal from the TA muscle was amplified, full-wave rectified and low-pass filtered at 100 Hz prior to averaging. The responses to the stimuli were analyzed on-line. The step cycle was divided into sixteen equal segments in time, starting from the time of ipsilateral heel contact. The EMG responses resulting from stimuli that occurred in each of the sixteen time segments were averaged together. A minimum of twenty responses were averaged for each time segment. The duration of this recording session was approximately 15 min. Results from this part of the experiment provided estimates of the time in the step cycle when the facilitatory and the suppressive responses were observed. This allowed us to target these two times in the step cycle in subsequent trials (see below).

In the second part of the experiment, intramuscular electrodes were used to follow the firing behaviour of single motor units. The stimulator was triggered after one of two fixed delays from the time of ipsilateral heel contact, so that either facilitatory or suppressive responses would be generated (as determined in the first part of the experiment). Stimuli were applied randomly to these two phases of the step cycle, about once every three steps.

This allowed us to follow the firing pattern of the same motor units in steps that received stimuli and those that did not. The recording was performed continuously for about 45 min to provide a minimum of 200 stimuli in each phase of the step cycle. Two hundred stimuli were found necessary to characterize the post-stimulus time histogram adequately. A gradually increasing static contraction was performed prior to and after each walking sequence to verify the presence of motor units in the recording. All data were stored on VCR tape for off-line analysis (bandwidth DC to 7 kHz).

Surface EMGs were also recorded from five of the subjects while they walked at their normal speed and with their normal footwear, as well as at the slower speed used in this experiment with the splint on. This allowed us to determine the effect of wearing the splint on the pattern of activation of the TA muscle.

2.2.4 Data analysis

All data originating from the second part of the experiment were analyzed off-line. Single motor units from the intramuscular EMG recording were discriminated by the shape of their unit potential (8701 Waveform Discriminator from Signal Processing Systems, Prospect, Australia). Slight variation in the shape of a unit potential was allowed. The permitted amount of variation was chosen to prevent inclusion of other units or movement artifacts. The time of appearance of the motor unit potential and the shape of the potential was carefully examined prior to digitization. The shape discrimination program generated a short pulse each time a single unit was recognized. This short pulse was delayed by a few milliseconds (4-10 ms) relative to the real time of occurrence of the spike, because of the processing time required to perform the shape discrimination. This delay was set by the

experimenter for each unit, and remained constant for that unit. These delays have been corrected for in all of the figures shown.

The surface EMG, footswitch and short pulses corresponding to the firing of single motor units were digitized off-line at 300 Hz, using the Axotape Data Acquisition System (Axon Instruments Inc., Foster City, CA, USA) and stored on disk. Averages of the surface EMG were calculated for steps that received stimuli (disturbed steps) and those that did not (undisturbed steps), separately. The averaging process was triggered by the time of heel strike in the case of the undisturbed steps and by the stimulus pulse in the case of the disturbed steps. In this latter case, data from the two different phases of the step cycle, the middle of the swing phase and the transition from swing to stance, were averaged separately. The averaged EMG data from undisturbed steps were then subtracted from the EMG obtained during disturbed steps to generate the responses to stimulation. Histograms indicating the probability of firing of single motor units were also calculated separately for both disturbed and undisturbed steps. The bin width of the histograms was 7 ms. The number of occurrences of a unit in any one bin was divided by the total number of steps averaged to give the probability of firing of that unit for that bin. Histograms were digitally filtered at 15 Hz (MATLAB software from MathWorks Inc., Natrick, MA, USA), in both the forward and reverse directions so that there was no time lag introduced. The histogram from the average of undisturbed steps was subtracted from those obtained during disturbed steps to generate the change in firing behaviour as a result of the stimulus.

The instantaneous firing rate of single motor units was also calculated. Whenever a motor unit fired, a search was made for the previous occurrence of firing of the same motor unit. If these two occurrences were within the same EMG burst in the step cycle, then the

instantaneous firing frequency was calculated from the time interval between the occurrence of the two spikes. If these two occurrences spanned the two EMG bursts during walking, then this interval would be discarded. In this way, firing frequencies were estimated for each of the EMG bursts separately.

2.3 RESULTS

2.3.1 Effect of wearing a splint and walking at a slower speed

Walking at a slower speed with a splint that limited the range of motion at the ankle joint to about 50% that of normal had some effect on the activity level of the TA muscle. The amplitude of EMG activity in the TA muscle during the swing phase, when subjects walked at a slower speed with the splint on, was an average of 89% that of the subjects walking at a normal speed with normal footwear (one example is shown in Fig. 2.2). The main difference was seen during the transition between the swing and the stance phases. The amplitude of the EMG when walking with the splint was an average of 71% of that during normal walking at this time in the step cycle (Fig. 2.2). Some subjects failed to clearly activate the TA muscle at the time of transition from swing to stance when wearing the splint. Activity at the transition from the swing to the stance phase was needed to test the hypotheses of this study. Subjects who failed to exhibit the normal biphasic activity in the TA muscle could not be included in this study.

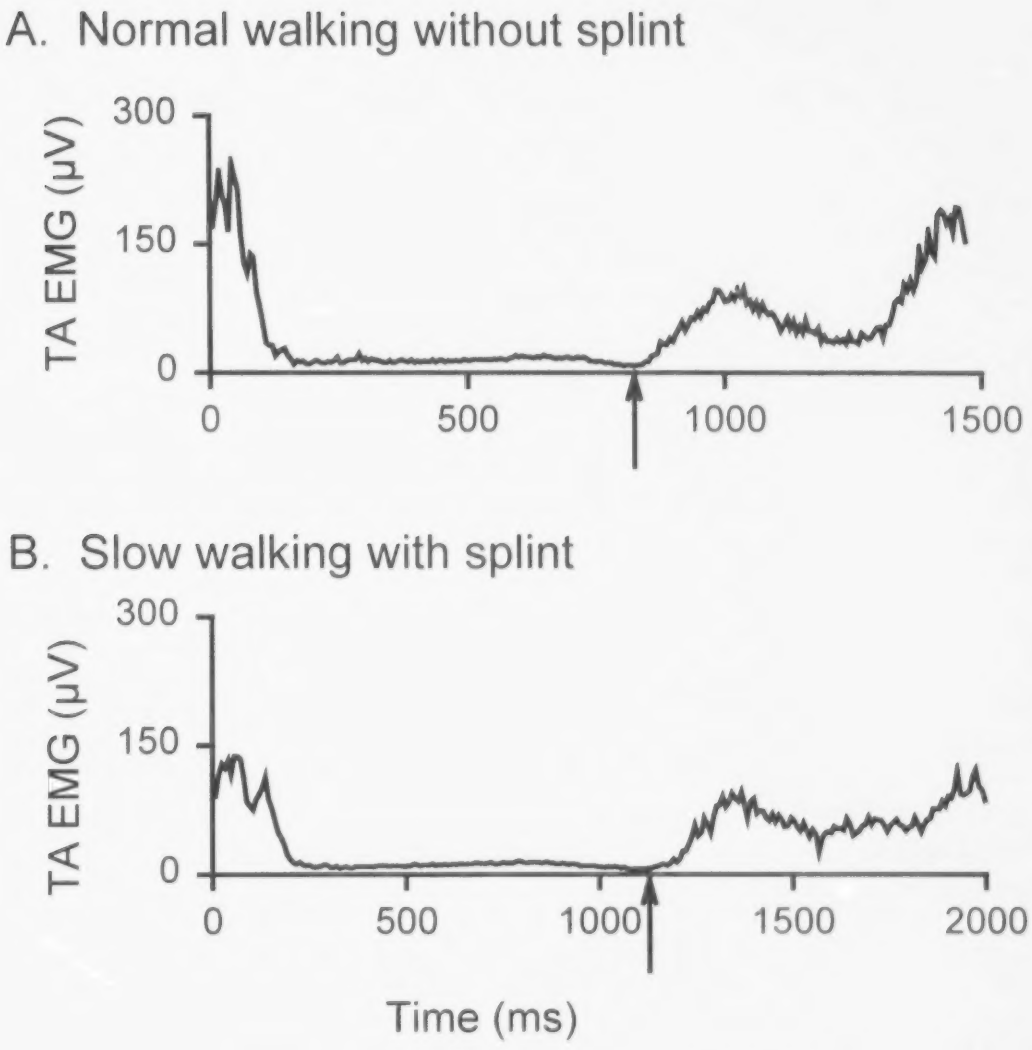

Figure 2.2 - Averaged surface EMG from the TA muscle during walking
Averaged surface EMG (n=51) from the TA muscle during walking, from one subject, displayed in time from heel contact of the right foot to heel contact again on the same foot. *A*. The EMG pattern when the subject was wearing his normal footwear and walking at a normal speed (1.11 m/s). There are two bursts of activity, one in the swing phase and a larger burst at the transition from swing to stance. *B*. The EMG pattern when the subject was wearing the splint and walking at a slower speed (0.62 m/s). Note that when wearing a splint, the amplitude of the EMG is reduced by about 10% in the swing phase, and by about 54% at the transition from swing to stance, when compared to the normal pattern in this subject. The arrows indicate the time at which the toe is lifted off the ground.

2.3.2 Methodological problems

The phase in the step cycle during which a suppressive response is seen is usually very short (see Yang & Stein, 1990). In most cases, this suppressive response was present in only one of the sixteen time segments in the step cycle, which corresponded to a time period of 85-95 ms at the walking speeds used in this study. This meant that in order to target this short phase for the generation of post-stimulus time histograms, the occurrence of the stimulus had to be very well timed to the step cycle. Stimuli were applied after a fixed delay from the time of heel contact. Hence, the subjects had to maintain a very consistent rate of walking over the 45 min time span required for the data collection. This was difficult for some subjects. The absence of an overt reversal in the reflex in some subjects could have been related to variations in the duration of the step cycle.

A small number of subjects (7 out of 26) failed to show a clear reflex reversal. It is known that the reversal is seen at certain stimulus intensities only (J.F. Yang & R.B. Stein, unpublished data). High stimulus intensities tend to generate facilitatory responses in both phases (see also Crenna & Frigo, 1984; Bélanger & Patla, 1987). In these subjects, however, a reversal was not seen at a variety of stimulus intensities, ranging from 1.3 to 1.7 times motor threshold. Rather, a modulation of the facilitatory or suppressive response was observed. The proportion of subjects that did not exhibit a reversal is similar to that reported by Duysens, Tax, Trippel and Dietz (1992). Most of the subjects in the current study who did not show a reversal showed facilitatory responses, whereas those from the previous study (Duysens *et al.* 1992) showed mostly suppressive responses. The reason for these differences are unclear. Subjects who did not show a reflex reversal could not be included in the present

study. As a result of these difficulties encountered in the experiments, and others described in the DISCUSSION, only twelve subjects provided good data sets.

2.3.3 Motor unit behaviour in undisturbed walking

Data obtained from the undisturbed steps in three subjects are shown in Fig. 2.3. The probability of firing of single motor units at different times in the step cycle is represented in the histograms of Fig. 2.3 (bottom trace for each subject). The averaged surface EMG pattern is shown in the top trace for each subject. Fig. 2.3*A* and *B* shows units that were recruited during both bursts of activity of the TA muscle. Among the forty-six units that were recorded overall, forty-three of them (93%) fired during both bursts of EMG activity. For each of these forty-three units, the firing pattern during walking was comparable to the EMG activation pattern of the muscle, recorded with the surface electrodes. Three motor units showed firing during one burst of EMG activity primarily and they were mostly active during the swing phase rather than at the transition from swing to stance, although a very limited amount of activity was evident in the transition phase for two of these units. An example is shown in Fig. 2.3*C*. Averaged data from all subjects during the unstimulated steps showed an averaged firing frequency of 21 Hz for the swing phase, and 19 Hz for the transition from swing to stance.

2.3.4 Responses to cutaneous stimulation

The averaged response of the TA muscle to tibial nerve stimulation for two different segments of the step cycle is shown in Fig. 2.4. The background EMG activity obtained from undisturbed walking has been subtracted in order to isolate the reflex response. A facilitatory

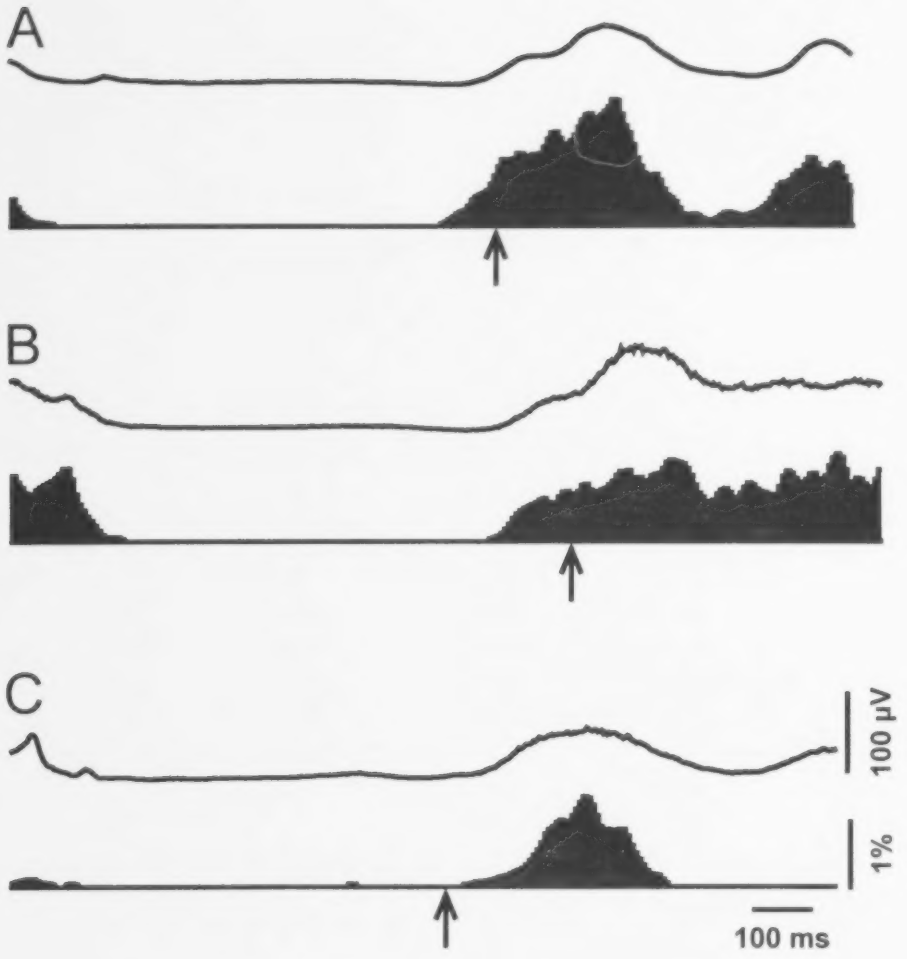

Figure 2.3 - Surface EMG and firing behaviour of single units during undisturbed walking

The averaged surface EMG from the TA muscle (top trace for each subject) shows the typical two-burst pattern, with the burst at the transition from swing to stance being slightly lower in some subjects. The probability of firing of single motor units (bottom trace for each subject) follows the profile of the surface EMG in parts *A* and *B*. The majority of motor units from which recordings were made showed activity during both bursts of TA activity. *C* shows an example of a unit that fired primarily in one burst. *A*, *B* and *C* are plotted on the same scale (amplitude and time scales shown in *C*). The arrows indicate the time at which the toe is lifted off the ground.

response at a latency of 60-70 ms after the onset of the stimulation is seen in the surface EMG when the stimulus was applied in the middle of the swing phase (Fig. 2.4*A*). A suppressive response at approximately the same latency is seen when the stimulus was applied at the transition from swing to stance (Fig. 2.4*B*). Histograms showing the probability of firing of three motor units from two subjects are also shown for both phases. The firing of unit 1 was facilitated in response to the stimulus applied in the middle of the swing phase and was suppressed when the stimulation occurred at the transition from swing to stance. Units 2 and 3 were recorded for the same subject at two different sites in the muscle. The suppression of the EMG activity at the transition from swing to stance was much smaller than the facilitation in middle of the swing phase in this subject. Nevertheless, the histograms showing the probability of firing of a single motor unit in response to the cutaneous stimulus mirrored the findings in the surface EMG. These units were recruited at low (unit 2) and moderate (units 1 and 3) levels of static contraction. It was possible to follow three units from two different subjects for the length of time required to obtain these histograms. All three units exhibited similar responses.

In Fig. 2.4*B*, the suppression at a medium latency is preceded by a small amount of facilitation. To rule out the possibility that this suppression is a result of the unit being refractory following the facilitation, the histograms were recalculated in the following way. When generating the post-stimulus time histograms, a check was incorporated in the software to exclude any trials in which the unit fired during the period when an early facilitation was observed. In this way, if a suppression was still seen at the later time interval, it could not have been a result of the unit being refractory. A clear suppression was still seen in each case at the later latency, and indeed remained largely unchanged. This was the case

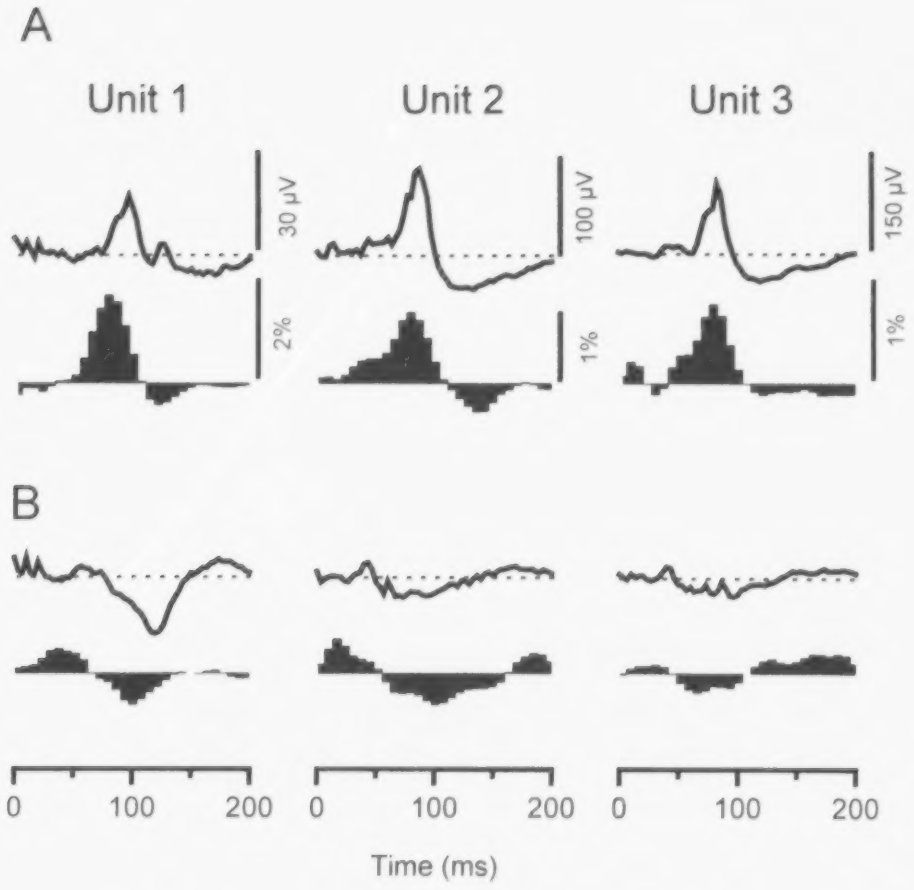

Figure 2.4 - Averaged surface EMG from the TA muscle and the post-stimulus time histogram of three motor units for two different segments of the step cycle

A. Averaged surface EMG from the TA muscle and the post-stimulus time histogram of three motor units, in response to stimuli applied during the middle of the swing phase, 1.03 s (unit 1), and 1.09 s (units 2 and 3) after heel contact. The same data recorded at the transition from swing to stance is shown in *B*, which was recorded at 1.42 s (unit 1) and 1.4 s (units 2 and 3) after heel contact. Note that in both cases, the profile from undisturbed walking has been subtracted to show the effect of stimulation. The surface EMG shows the typical reversal observed, facilitation in the swing phase and suppression at the transition from swing to stance. The post-stimulus time histogram shows that the firing pattern of a motor unit is facilitated during the swing phase and suppressed during the transition from swing to stance.

for all three units for which post-stimulus histograms were generated. Hence, it confirms that the suppression was not a result of the preceding facilitation.

2.4 DISCUSSION

The results indicate that: (1) most motor units were active during both phases in the walking cycle when the TA muscle was active, and (2) in response to the stimulus, motor units which were facilitated in the swing phase were suppressed during the transition from swing to stance. These results suggest that the phase-dependent reversal of the sign of a reflex during human walking can be explained by the hypothesis that there are parallel interneuronal pathways from cutaneous afferents to single motoneurones. These parallel pathways are alternately favoured as a function of the walking cycle.

2.4.1 Methodological considerations

Recording from single motor units during human walking is technically very difficult. It has been reported by one other research group only (Grimby, 1984; Jakobsson, Borg, Edström & Grimby, 1988). Many problems were encountered during these experiments which resulted in a high failure rate. The most difficult problem was the movement of the intramuscular electrode tip within the muscle during walking. In many cases, units present at the beginning of the recording sequence would disappear, and new units would appear. These units could not be used to generate the post-stimulus time histograms, but were often still

useful in determining the firing behaviour during unstimulated steps, as long as a minimum of 200 undisturbed strides were recorded.

The identification of single motor units was based on the shape of the action potential recorded. Is it possible that two motor units with very similar shapes, each firing during one phase of the step cycle only, were considered the same unit? Although this possibility cannot be entirely ruled out, we feel that this situation must have been rare. We were careful to select only those units that exhibited either large amplitudes or unique shapes. In this way, it is very unlikely that two units with identical complexities in their shape were being recorded at the same time (see Jakobsson *et al.* 1988).

Is there any chance we could have sampled a limited type of motor unit, and thus have missed different behaviours in other motor unit types? Two factors should be considered in this context. First, the slower speed of walking and the use of a splint may have required considerably less activity of the TA muscle. This may have limited our observations to the smaller motor units only. The EMG amplitude recorded from the TA muscle in the current experimental condition was not substantially different from that in normal walking. Second, the location at which motor units were sampled in the muscle was close to the origin of the muscle, because the recording was more stable there. If there are large differences in the motor unit types in the proximal compared with more distal regions of the muscle, our sample could have been biased. Muscle fibres appear to run the full length of the fasciculi in the TA muscle (Feinstein, Lindegård, Nyman & Wohlfart, 1955). There is no evidence to suggest that proximal-distal differences exist, particularly for muscles that appear to be functionally homogeneous, such as the TA. Even in muscles containing functionally distinct subpopulations of motor units, a proximal-distal separation of function is rare (Pratt &

Loeb, 1991). There is limited evidence for histological regionalization in the TA muscle in the human (Henriksson-Larsén, Lexell & Sjoström, 1983; Henriksson-Larsén, 1985), but this appears to be a difference between superficial and deep regions of the muscle. Since recordings of single motor units were obtained throughout superficial and deep sections of the muscle in this study, it is unlikely that the motor units sampled represented a biased sample.

2.4.2 Firing behaviour of single motor units

Motor units recorded in these experiments showed firing rates that averaged around 19-22 Hz. This is very similar to that reported by Jakobsson *et al.* (1988) for the same muscle. In the study by Jakobsson *et al.* (1988), however, there was a greater difference in the firing rate of motor units between the two bursts of activity during walking. Firing rates were higher for the burst at the transition from the swing to the stance phase (20-25 Hz) than for the burst during the swing phase (10-15 Hz). This is probably related to the footwear. Wearing the splint tends to lower the EMG amplitude during walking, particularly during the burst at the transition from swing to stance. In most subjects, the two bursts of EMG activity were either roughly the same amplitude (Fig. 2.2), or the burst during the swing phase was slightly larger (Fig. 2.3*A* and *B*). For that reason, large differences in the firing rate of individual motor units during the two phases of activity was not expected.

2.4.3 Neural mechanisms

COULD THE REVERSAL HAVE RESULTED FROM TWO SUBPOPULATIONS OF MOTOR UNITS IN THE TA MUSCLE? The majority of motor units in the TA muscle were recruited

twice during the walking cycle. This indicates that the two bursts of TA EMG in walking do not represent two separate subpopulations of motor units. Three of the forty-six motor units recorded from fired in one part of the step cycle only. These three motor units most likely had higher recruitment thresholds than the others, since they tended to be recruited in the phase of walking when the amplitude of the EMG burst was larger. The conclusion that there are no functional task groups within the TA muscle during walking is in agreement with other studies of this muscle in both humans and cats (Jakobsson *et al.* 1988; Pratt, Chanaud & Loeb, 1991). The TA muscle is a mechanically simple muscle. It appears that functional task groups exist in muscles that are mechanically complex and subserve more than one action (Hoffer, Loeb, Sugano, Marks, O'Donovan & Pratt, 1987; Chanaud, Pratt & Loeb, 1991; Pratt & Loeb, 1991).

Although most motor units fired in both bursts of TA activity during walking, there is still the possibility that the reflex reversal is generated by two populations of motor units. Fig. 2.1*B* illustrates that a group of afferents may synapse onto two populations of motor units, exciting one group and inhibiting another (Burke, Jankowska & Ten Bruggencate, 1970; Kanda, Burke & Walmsley, 1977; Nielsen & Kagamihara, 1993). Both groups of motor units are active during the swing phase and the transition from swing to stance. The reversal in the reflex could result from alternate gating of the pathways as a function of the step cycle. This mechanism, however, is not likely responsible for the reversal seen here. The reversal was seen in the behaviour of a single motor unit; that is, the firing of single motor units were facilitated by cutaneous input in one phase of the step cycle and suppressed by the same stimulus in another phase (Fig. 2.4).

THE REVERSAL MOST LIKELY RESULTED FROM PARALLEL PATHWAYS TO SINGLE MOTOR UNITS. The best explanation for the current results is that there are parallel pathways from cutaneous afferents to single motor units: some pathways are excitatory, some inhibitory. There is a gating or a shift in balance between the two pathways during walking, such that excitatory pathways are dominant during the swing phase, and inhibitory pathways are dominant during the transition from swing to stance. We favour the explanation that there is a shift in the balance between the two pathways rather than a gating mechanism, because some subjects do not exhibit a clear reversal, but rather a modulation of the facilitatory response. Perhaps the presence of a reversal is dependent on the strength of the bias formed between the two pathways.

The physiological correlates to support such a mechanism clearly exist. There is ample evidence for the existence of both excitatory and inhibitory pathways to single motoneurones (Eccles & Lundberg, 1959; Schomburg & Steffens, 1986) from cutaneous and muscular afferents. There is also evidence that other inputs, particularly supraspinal, can favour specific interneuronal pathways (Lundberg & Voorhoeve, 1962; Hongo, Jankowska & Lundberg, 1969; Fleshman, Rudomin & Burke, 1988).

The modulation or gating could occur at many levels of the reflex pathway including the terminals of the primary afferents, the interneurones in the reflex pathway, and the motoneurones (reviewed in Rossignol *et al.* 1988). The current results suggest that the motoneurone is not the primary site for generating the type of reversal observed here. The importance of other sites cannot be determined in the human at the present time. Evidence from animal work suggests that the interneurone is a probable site for this type of reversal. Convergence of input from the central pattern generator for locomotion and from sensory

afferents produces reflex modulations in lower vertebrates (Sillar & Roberts, 1988, 1992). Convergence of this type has also been reported in cats (Edgley & Wallace, 1989; Shefchyk, McCrea, Kriellaars, Fortier & Jordan, 1990).

Whatever the mechanisms for generating the reversal, the results reported here indicate that the control must be very specific. Two parallel pathways projecting to the same motoneurone must be controlled differentially at different times in the step cycle to produce these effects. Such specificity in control has been reported for pathways converging onto single motoneurones during fictive locomotion in the cat, although these pathways subserved afferents from different nerves (Moschovakis, Sholomenko & Burke, 1991). The current study suggests that pathways activated by the same peripheral nerve can also be controlled in this way.

Post Scriptum

This chapter has already been published:

DE SERRES, S.J., YANG, J.F. & PATRICK, S.K.
Mechanism for reflex-reversal during walking in the human tibialis anterior muscle as revealed by single motor unit recording.
***Journal of Physiology* 488, 249-258 (1995)**

I was involved in this project from the beginning and worked on every step of its realization: experimental setup preparation (including the design and fabrication of intramuscular electrodes), data collection, data analysis (including writing computer programs to perform the analysis; see APPENDIX B), and preparation of the manuscript.

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CHAPTER 3

REGULATION OF SOLEUS MUSCLE SPINDLE SENSITIVITY IN DECEREBRATE AND SPINAL CATS DURING POSTURAL AND LOCOMOTOR ACTIVITIES

3.1 INTRODUCTION

Stretch reflex responses are highly modulated during locomotor activities in decerebrate cats (Akazawa, Aldridge, Steeves & Stein, 1982). The averaged stretch reflex in spinal cats is markedly lower during locomotion, compared to the resting state just prior to locomotion (Chapters 4 and 5). A possible contribution to this lowered stretch reflex is an increased activation of static γ-motoneurones (static γ-drive), which can reduce spindle sensitivity to stretch (Jansen & Matthews, 1962; Taylor, Stein & Murphy, 1985). Indeed, in decerebrate cats muscle spindle sensitivity is on average lower during locomotion compared to the pre-locomotor state, in spite of large increases in firing rates (Taylor *et al.* 1985). Direct recordings from γ-motoneurones in these cats support the idea that a strong static γ-drive is initiated during locomotion (Murphy, Stein & Taylor, 1984; Bessou, Cabelguen, Joffroy, Montoya & Pagès, 1986). In contrast, the dynamic γ-drive is not increased on average during locomotion (though it is phasically modulated with the EMG; Cabelguen, 1981; Murphy *et al.* 1984; Bessou *et al.* 1986). Recordings from muscle spindle

afferents during locomotion in intact cats also show a much reduced sensitivity to the ongoing muscle movements (Prochazka, Hulliger, Zangger & Appenteng, 1985; Hulliger, Dürmüller, Prochazka & Trend, 1989), suggesting that the increased static γ-drive seen in decerebrate cats is also a part of the normal functioning of the intact locomotor circuitry.

It is unclear whether this increased static γ-drive arises from locomotor circuitry in the spinal cord, or supraspinal structures. In the only study of afferents of spinal locomoting cats performed to date (Sjöström & Zangger, 1976) γ-motoneurones were strongly driven during locomotion induced by DOPA. However, in this study there was only tentative evidence that this was static γ-drive in flexor motoneurones, and the identity of the γ-drive was unknown in extensors. In contrast, the work of Lundberg's group gives a clear indication that in spinal cats static γ-motoneurones are strongly driven after DOPA administration (in flexors: Grillner, Hongo & Lundberg, 1967; Bergmans & Grillner, 1968,1969; and in extensors: Grillner, 1969), but locomotion was not produced in these cats. However, their results may be relevant, since they felt that the long latency flexor reflexes that they studied (FRA reflexes) were a manifestation of single steps from the spinal locomotor rhythm generator (Jankowska, Jukes, Lund & Lundberg, 1967; Gossard & Hultborn, 1991).

We thus sought to determine if the reduced stretch reflex in the ankle extensor muscles of the spinal locomoting cat might be in part explained by an increased static γ-drive to muscle spindle afferents. Since the reflex responsiveness is also reduced in the decerebrate cat during crossed extensor reflex activation (Chapters 4 and 5) and during spontaneous tonic contractions (Akazawa *et al.* 1982), we also studied this state to determine if the changes in γ-drive are specific to locomotion. The net γ-drive was estimated from changes in the

sensitivity of muscle spindle afferents, as described by Taylor *et al.* (1985). We found that usually a strong static γ-drive was initiated with the onset of either locomotion or crossed extensor reflex contractions. However, there was some variability in this static γ-drive related to the pharmacological method of initiating locomotion. When locomotion was initiated by administration of DOPA, the γ-drive was modulated phasically with the EMG. In contrast, when Clonidine was administered, the γ-drive was absent in a third of the units, in spite of locomotor contractions. The remaining units received tonic γ-drive, not modulated with the EMG.

3.2 METHODS

Acute experiments were performed on 15 decerebrate cats (3-3.5 kg) to investigate the firing of soleus muscle spindle afferents in various states. Initially the effects of tonic contractions induced by crossed extensor reflex stimuli were evaluated (11 cats). The cord was then transected at the thoracic level (T12) and this spinal state was investigated with locomotion induced pharmacologically with Clonidine (6 of the 11 cats). Finally, in all cats the firing of the muscle spindle afferents was recorded following removal of the γ-drive by cutting the ventral roots (L6-S1, deefferented state). Also, in 3 of the 6 Clonidine-treated cats the effect of the drug was studied in the decerebrate state, prior to spinalization. In an additional 5 cats, locomotion was investigated in the spinal state with DOPA used instead of Clonidine.

3.2.1 General preparation

Under general anaesthesia (Halothane (1.5-2.5%) mixed with 95% O_2, 5% $C0_2$) both hindlimbs were extensively denervated (to eliminate unwanted movement), sparing only the nerves supplying the soleus on the ipsilateral side and the triceps surae on the contralateral side. In addition, on the ipsilateral side nerves of the hip area were cut where they branched from the sciatic above the hamstrings. Nerve cuffs were placed on the ipsilateral sciatic nerve and the contralateral common peroneal (CP) nerve (the latter for crossed extensor reflex activation with 0.2 ms pulses at 50 Hz, with intensity graded to produce different contraction levels). The soleus muscle was cut from its distal insertion and freed from connective tissue. A laminectomy was performed at L7-L5, and T12. The cat was placed in a stereotaxic apparatus and a premammillary decerebration was performed (cut made precollicular at 45^0), after which the anaesthetic was removed. EMG electrodes were implanted in the soleus and the contralateral triceps surae muscles. The ipsilateral knee and foot were rigidly fixed and the soleus was tied to a muscle puller (310B, Cambridge Technology Inc., Watertown, MA, USA) at optimal length (peak of length-tension curve; equivalent to ankle flexion of 135^0; Rack & Westbury, 1969). The contralateral leg hung free, and moved spontaneously during locomotion. A paraffin pool was formed with the skin flaps over the laminectomized area, and maintained at 35-37^{0}C with radiant heat. The soleus muscle was covered with skin and also maintained at 35-37^{0}C with radiant heat.

3.2.2 Drugs

Locomotion was facilitated in the spinal state by intravenous administration of the α_2 noradrenergic (NA) agonist Clonidine (50-200 mg/kg; Sigma Chemical Co., St.Louis, MO,

USA), preceded by treatment with the opiate antagonist Naloxone (25-50 mg/kg; Sigma Chemical Co.; see Pearson, Jiang & Ramirez, 1992). Additional manual stimulation of the perineal region was required to produce locomotion. Locomotion was also produced by L-DOPA (50 mg/kg; Sigma Chemical Co.) in spinal cats pre-treated with the monoamine oxidase inhibitor Nialamide (50 mg/kg; Sigma Chemical Co.). Likely DOPA is taken up acutely by the NA terminals, and displaces endogenous noradrenaline. This noradrenaline then remains available for hours because of the Nialamide, and acts on both α_1 and α_2 NA receptors; additional DOPA is rarely effective, probably because the initial dose depletes the NA supplies (H. Hultborn, personal communication). Clonidine usually decreased and DOPA increased the blood pressure. Blood pressure was kept above 60 mmHg with Dextran 40 (100 mg/ml; Baxter Corporation, Toronto, Canada) or occasionally epinephrine, especially during Clonidine infusion. Recordings were taken once the blood pressure was stabilized. Two spinal Clonidine-treated cats were pre-treated before surgery with the cholinergic antagonist atropine (0.2 mg; Sigma Chemical Co.) to prevent respiratory congestion. This was probably ineffective by the time of recording (about 8 hours later).

3.2.3 Dorsal root filament identification

Afferents were recorded from the distal ends of small filaments cut from either S1 or L7 dorsal roots. Filaments were divided until only one or two discriminable soleus afferents remained, which was possible because of the extensive denervation. Afferents were classified as group Ia, Ib or II by using standard ramp, vibration and twitch tests (Matthews, 1972). Conduction velocity, measured by the afferent impulse triggering an average of the sciatic nerve cuff signal, was used to confirm the identification of group I vs. group II afferents

(70 m/s as the dividing line; there were no ambiguous units at this dividing line). Afferent responses were recorded on tape during the experiments, and digitized afterwards with an Axotape system (Axon Instruments Inc., Foster City, CA, USA). Discrimination of the units was performed with a window discriminator (Bak DIS-1, Bak Electronics Inc.). Afferent firing rate was viewed on-line with an instantaneous rate device (Bak ISI-1, Bak Electronics, Inc.). In Figs. 3.3, 3.6, 3.8 and 3.10 described below, the firing rate in the interval between spikes was fixed at the rate determined for that interval (zero-order-hold interpolation). This gave a continuous signal which was filtered (50 Hz lowpass) for visualization of the 4 Hz modulation to stretch.

3.2.4 γ-drive estimation from stretch

To estimate the relative contribution of static and dynamic γ-drive, 4 Hz, 0.25 mm sinusoidal stretches were applied to the soleus. The step cycle was divided into 8 phases and stretches occurring in a given phase were used to produce a post stretch triggered histogram (PSTH) as follows (see also Fig. 3.1): The sinusoidal stretch cycle (250 ms) was divided into 16 equal periods (bins) and the number of afferent spikes in each bin was counted from all stretches. The firing rate was computed by dividing the total number of spikes per bin by the number of stretches in the average and by the bin duration. The total γ-drive to a Ia unit was inferred from the mean rate of firing during the stretch (mean of PSTH). The non-stretch related trend in the PSTH (due to fluctuations in firing rate during locomotion) was then subtracted. This trend was computed by repeating the above step cycle PSTH estimation with an artificial sample of stretch cycle markers uncorrelated with the actual stretch. This procedure was verified by simulations. The sensitivity was estimated from the amplitude of a

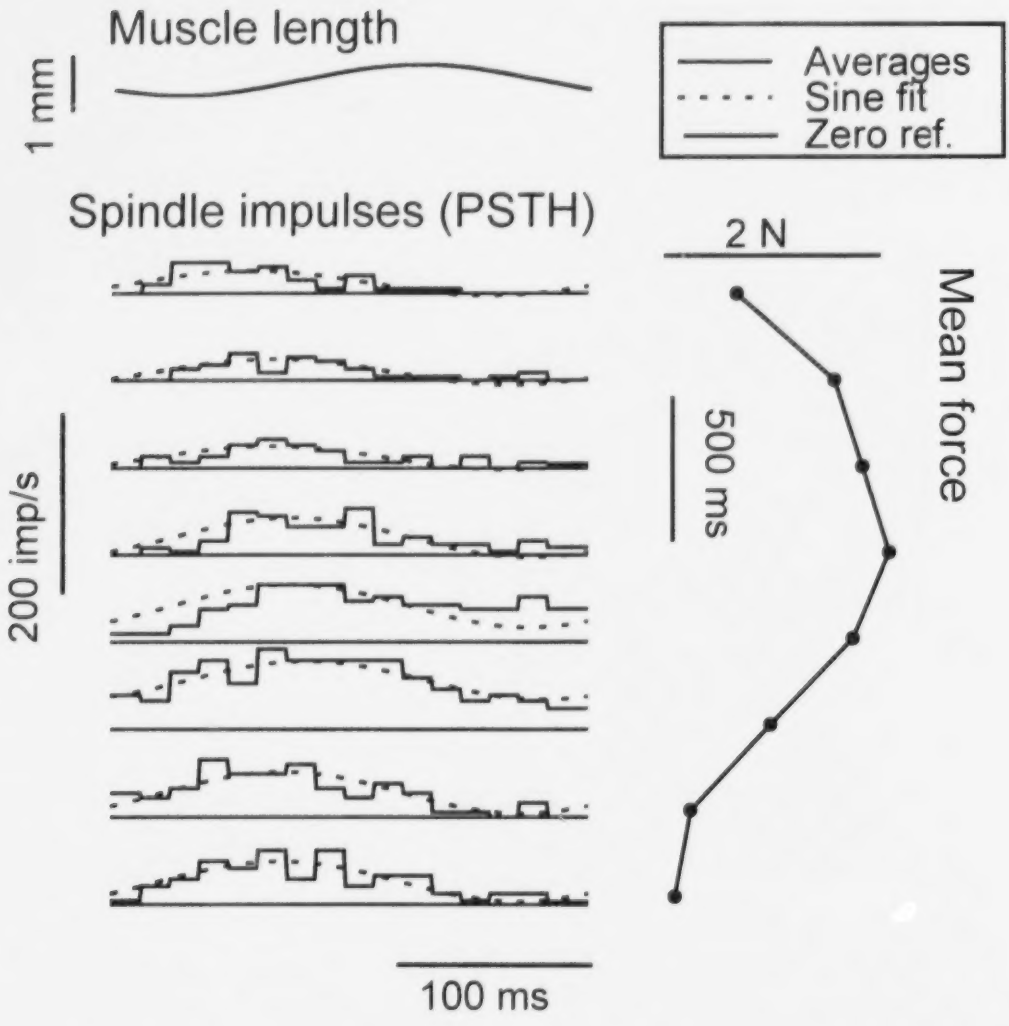

Figure 3.1 - Method for averaging soleus afferent firing during different step cycle phases.

Upper panel: averaged imposed stretch. Lower panel: step cycle dependent afferent firing. Steps were divided into 8 equal periods. Stretches and afferent impulses that occurred in each of the 8 periods of all steps were used to construct post stretch triggered histograms (PSTHs), giving the afferent response to stretch for each period. Averaged muscle force in each period is shown by open circles plotted in a perpendicular orientation to the right. Dotted lines: best fit sine waves. Averages taken from 23 step cycles of the unit shown in Fig. 3.3*B* (see RESULTS). Positive length changes correspond to muscle stretch, as in all subsequent figures.

sine wave fitted to the modulation in firing (dotted lines in Fig. 3.1; fitted optimally with standard linear least squares techniques, Norton, 1986; sensitivity equals modulation depth divided by stretch amplitude). Step cycle duration did not affect this analysis as the cycle was always divided into 8 equal segments regardless of the duration, and the flexion and extension phases were usually a fixed proportion of the step cycle.

Since we used the afferent mean firing rate during stretch (rather than before stretch) as an estimate of its γ-drive, the maximum possible modulation in afferent firing produced by the stretch is, of course, always limited by the mean firing rate. Specifically, if an afferent fires only a very brief burst during a stretch cycle (e.g. a single spike) and then silences, the computed rate modulation can be shown to be twice the mean rate during stretch (2:1 ratio; dashed lines in Figs. 3.5, 3.7 and 3.9). Higher modulation/rate ratios are impossible.

The effects on afferent sensitivity during dynamic and static γ-motoneurone activations are opposite, as shown by the lines drawn in Figs. 3.5, 3.7 and 3.9 (with thick arrows), which are taken from values in Table 3.1 of Taylor *et al.* (1985; single γ efferent stimulation effects with stretch parameters of 4 Hz and 0.22 mm, corrected to 0.25 mm, preparation similar except for less denervation on the contralateral side). With dynamic γ-drive alone the units become silent during the shortening phase of the sinusoidal oscillation, but fire at high rates during lengthening, thus producing a large overall modulation in firing (sensitivity) during the sinusoidal stretching. Static γ-drive prevents this silencing and generally decreases the sensitivity.

An additional complication is that unloading and relengthening of the muscle spindle by extrafusal contractions cause changes in firing of the unit which have to be distinguished

from the γ-drive influence. We studied this issue in 11 of the deefferented units by characterizing the relationship between afferent sensitivity and firing rate without the concomitant influence of γ-drive (this presumably also eliminates β-fibre activation, though in soleus β effects are minimal, e.g. see Grill & Rymer, 1985). The range of firing rates produced during locomotion was mimicked by applying slow ramp length and force changes, as shown in Fig. 3.2*A*. Sinusoidal oscillations superimposed on these ramps were used to measure the sensitivity. As seen from the raw trace in Fig. 3.2*A*, the afferent sensitivity to sinusoidal stretch increased during the ramp when the overall firing rate increased - even though there was no γ-drive. For analysis, each ramp was treated like a step cycle during locomotion. Ramps were divided into 8 equal periods and stretch-related PSTHs were constructed in each period. Modulation to stretch (sensitivity) was computed from the amplitude of a sinusoid fitted to the PSTH. The increase in sensitivity with firing rate is summarized in Fig. 3.2*B* for the 11 deefferented units studied (with 8 points per unit). Note the remarkable consistency among the behaviour of the different units. A quadratic polynomial was fitted to this relation (solid line in Fig. 3.2*B*) in order to predict the deefferented sensitivity corresponding to a given firing rate.

Statistical significance was tested with a Student's t-test at the 95% confidence level. Population distributions were usually sufficiently Gaussian to use this simple test. The one exception was the Clonidine-treated spinal cat. In this case there was a bimodal distribution, which was treated by dividing the population into units with and without γ-drive (see RESULTS).

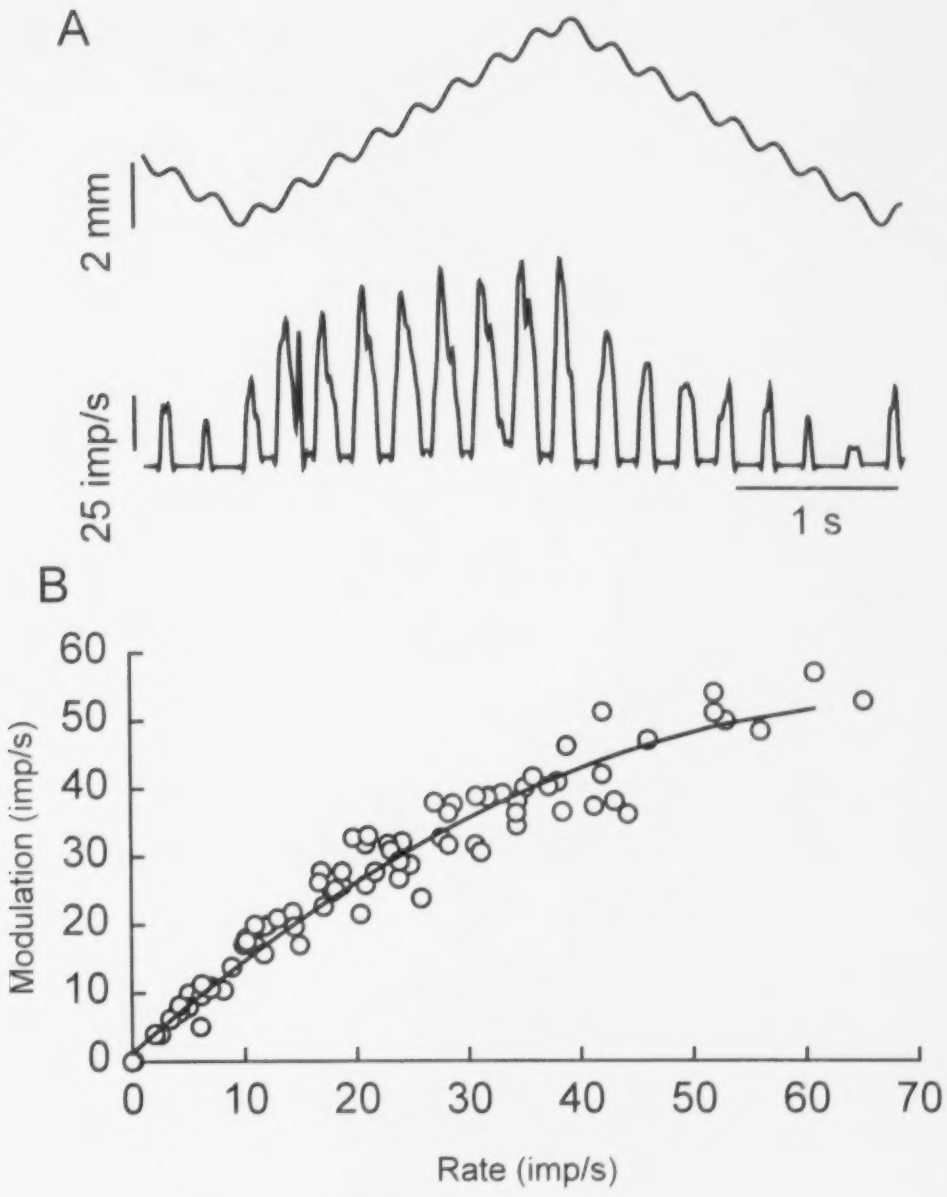

Figure 3.2 - Modelling of the effect of contraction-related spindle unloading.
A. Firing of a deefferented soleus primary afferent during the standard sinusoidal stretch superimposed on a ramp stretch to mimic the changes in firing rate during locomotion. The top trace represents the length signal and the bottom trace the firing rate of the unit. *B*. Modulation of 8 deefferented units (each as in *A*) at different mean firing rates caused by the slow ramp stretches (8 points per unit). Solid line, best fit quadratic polynomial. Note that the modulation to stretch increased with firing rate.

3.3 RESULTS

3.3.1 Clonidine-treated spinal cats

Resting state dynamic γ-drive. In Clonidine-treated spinal cats locomotor activity was rarely observed prior to perineal stimulation (Fig. 3.3*A*). In this resting state, primary afferents fired with a mean rate of 24±15 impulses/second (imp/s) and were modulated by 31±15 imp/s during the 0.25 mm sinusoidal stretch (mean±SD; n=18 units). This modulation corresponds to a sensitivity of 124 imp/s/mm (sensitivity equals modulation divided by stretch amplitude). In this paper we quote modulation values, rather than sensitivity, since they give a clearer indication of the actual firing rates during stretch; otherwise the terms modulation and sensitivity are used interchangeably in the discussions below (stretch was always 0.25 mm). With the dorsal roots intact the afferent discharge evoked by the stretch caused strong reflex EMG activation in this resting state (Chapter 4). However, in Fig. 3.3*A* such reflex EMG was not seen, likely because portions of the S1 and L7 dorsal roots were cut in searching for the afferents.

In the resting state the averaged firing rate and modulation during stretch were significantly above the corresponding values found after cutting the ventral roots (deefferentation; $p<0.05$), indicating that a *dynamic γ-drive* dominated (see also Fig. 3.5 described below). The deefferented units fired with a mean rate of 11±5 imp/s and had a stretch modulation of 17±4 imp/s (n=14 deefferented units; standard length; see Table 3.1). Not all units could be followed up to the time of deefferentation; thus, the averaged values come from 11 units from Clonidine-treated spinal cats and 3 units from DOPA-treated spinal

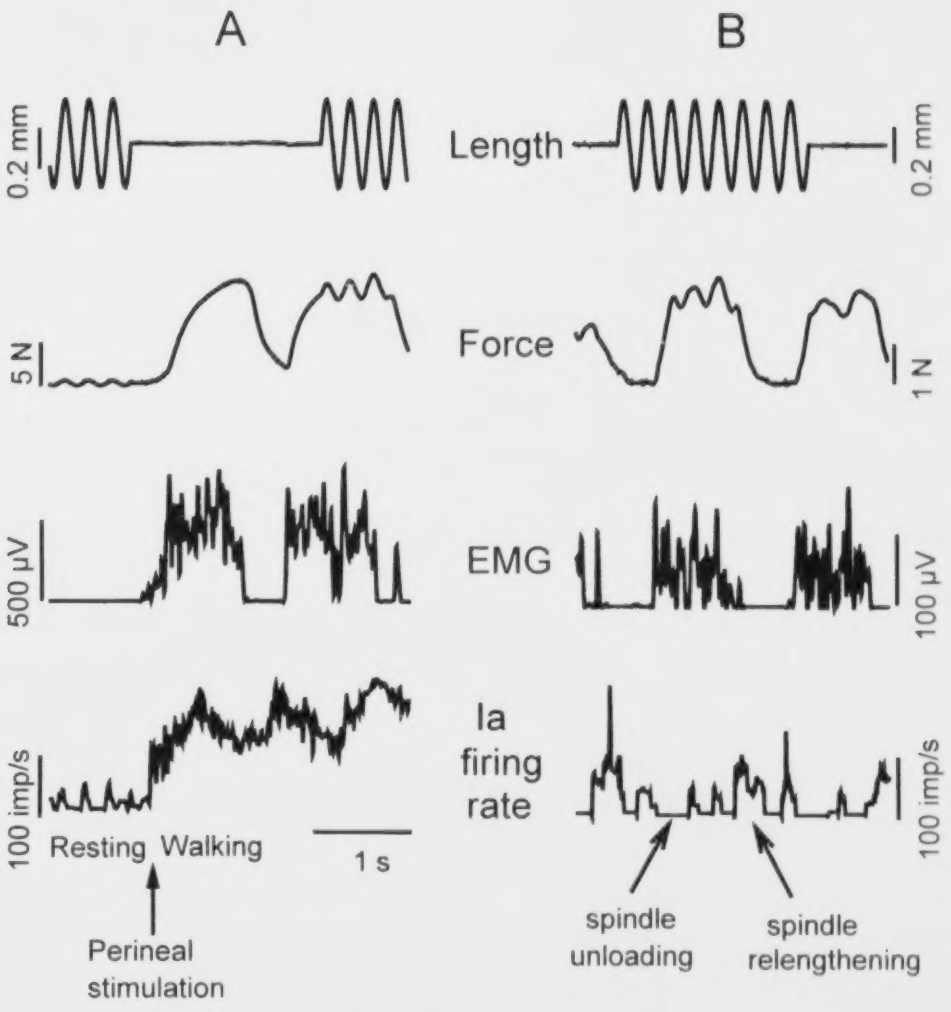

Figure 3.3 - Soleus primary muscle spindle afferents in Clonidine-treated spinal cats. *A*. Resting state before locomotion and transition into locomotion occurring at the onset of manual perineal stimulation (vertical arrow). Traces are from top to bottom: soleus length, force, EMG, and afferent impulse rate. The muscle was sinusoidally stretched 50% of the time around the standard length. During locomotion the soleus EMG was activated phasically (2 step cycles shown). Note that in the resting state (before arrow) the firing rate was low, but modulated by the stretch. During locomotion the rate was high and unmodulated by the stretch. *B*. 'Undriven' primary muscle spindle afferent recorded during locomotion in another cat. Note that the afferent responded to each cycle of the stretch, except when the muscle contraction started. Likely this was due to unloading of the spindle by the contraction, due to lack of γ-drive. When the contraction ended and the force dropped the unit fired maximally, due to relengthening of the spindle. Note that the locomotion in *B* was less vigorous than in *A* (smaller scale bars in force and EMG).

cats. No differences were found between the firing behaviour of the deefferented units in these two preparations; their responses were grouped together.

Condition	Spinal+Clonid. n=18		Spinal+DOPA n=10		Decerebrate n=18		Decer.+Clonid. n=7	
	Rate	Mod.	Rate	Mod.	Rate	Mod.	Rate	Mod.
Resting/no stim.	24±15	31±15	42±7	46±11	31±11	38±11	20±6	28±7
Flexion/no EMG	55±45	22±13	28±16	18±5	78±59	24±7	-	-
Extension/EMG	50±41	20±11	89±32	27±19	99±41	23±12	11±8	14±10
Mean flex.+ext.	53±42	21±10	59±19	23±9	89±45	24±7	11±8	14±10
Deefferented	11±5	17±4	-	-	-	-	-	-

Table 3.1 - Firing behaviour of primary afferents in spinal and decerebrate cats during rhythmic (locomotion) and tonic contractions.
Spinal cats treated with DOPA or Clonidine (Clonid.) to produce locomotion. Decerebrate cats untreated or treated with Clonidine and shown without (resting) or with crossed extensor reflex activation with CP stimulation. Firing shown without and with EMG associated with CP stimulation, and in mid-flexion and mid-extension during locomotion. Resting condition in spinal DOPA cats is prior to DOPA administration. Deefferented condition is from 14 units, and applies to all spinal and decerebrate cats. Oscillations 4 Hz, 0.25 mm. Sensitivity=modulation/0.25 mm. Firing rates and modulations (Mod.) in units of imp/s (mean±SD).

Walking state: static γ-drive. Since the soleus muscle undergoes large length changes during normal locomotion (>5 mm), muscle spindle firing patterns result from a complex mixture of γ-drive and mechanical lengthening effects (Taylor *et al.* 1985). To observe the effects of γ-drive in relative isolation we have studied the afferent firing during locomotor activity while holding the muscle at a fixed length, except for the test oscillations. The firing pattern of the primary afferent shown in Fig. 3.3*A* demonstrates one of the main findings of

this study. When locomotion commenced the firing rate was increased abruptly and remained high throughout the step cycle. Further, no clear modulation of the firing rate was present during sinusoidal stretching. This suggests that a *tonic static γ-drive* was introduced at the onset of locomotion (dynamic γ-drive may also have been slightly reduced; see below).

The γ-drive to the primary afferents was not always as strong as in Fig. 3.3*A*. A third of the afferents seemed to have little or no γ-drive, as shown in Fig. 3.3*B* (see also Table 3.2). These afferents had low mean firing rates, and although they responded to stretch, they were also silent during shortening. Further, at the onset of contractions they also silenced when the muscle was held at a fixed length or even stretched (see arrow labelled 'spindle unloading' in Fig. 3.3*B*). Note that although the total muscle length was held constant (or increased), the sarcomeres could still shorten internally and stretch the tendon. It is thus likely that the γ-drive was not sufficient to prevent the extrafusal contraction from unloading the muscle spindle by such internal shortening. Moreover, as the force fell at the end of contractions these afferents fired strongly, presumably due to the muscle spindle relengthening after the extrafusal contraction (Taylor *et al.* 1985; see arrow labelled 'spindle relengthening' in Fig. 3.3*B*).

The changes in afferent firing of Fig. 3.3*B* during a step cycle are seen more clearly in the averages shown in Fig. 3.1, described in the METHODS. The step cycle was divided into 8 equal periods, and the afferent response to stretch (PSTH) was computed in each period. The modulations to stretch were computed from the amplitude of the sine waves (dashed lines in Fig. 3.1) fitted to the PSTH responses, and are shown in the lower panel of Fig. 3.4.

From the third panel of Fig. 3.4, it is clear that the firing rate in this afferent was minimal when the contraction started, and maximal when the force dropped at the end of the contraction, likely due to unloading related to the contraction, as mentioned above. Thus, the firing rate was modulated completely out of phase (anti-phase) with the EMG (top panel of Fig. 3.4), as though the unit was without γ-drive. Moreover, if we assume that this unit was without γ-drive, we can predict its modulation to stretch (sensitivity) from the firing rate vs. modulation relationship described for deefferented units in METHODS (Fig. 3.2). The thin line in the lower panel of Fig. 3.4 shows this prediction, which appears to fit the actual data well, especially during the early part of the step cycle (extension) and at the points marked mid-flexion and mid-extension. Also, note that at mid-flexion and mid-extension the afferent was minimally influenced by the mechanical unloading effects; firing rates were close to the passive rate found after deefferentation (at the standard length; dotted lines), as discussed below. Other mechanisms, such as contraction-history dependence (Wilson, Gandevia & Burke, 1995) are unlikely to explain the anti-phase firing of these afferents.

Summary of Ia afferents. Fig. 3.5 summarizes the data from all afferents studied at mid-flexion and mid-extension, where the mechanical unloading and relengthening effects were likely to be minimal. At these points the rate of change of force was zero and thus the spindle internal velocity produced by contraction-related unloading was also low (though a small reduction in spindle length may have occurred during the mid-extension contractions, associated with about a 5 imp/s rate reduction, as in Fig. 3.4). Thus, afferents in Fig. 3.5 with firing rates that exceeded the deefferented firing rate (large cross near origin of Fig. 3.5) must have received γ-drive. This was the case for the majority of afferents, with the exception of the weakly driven units described above in Fig. 3.4.

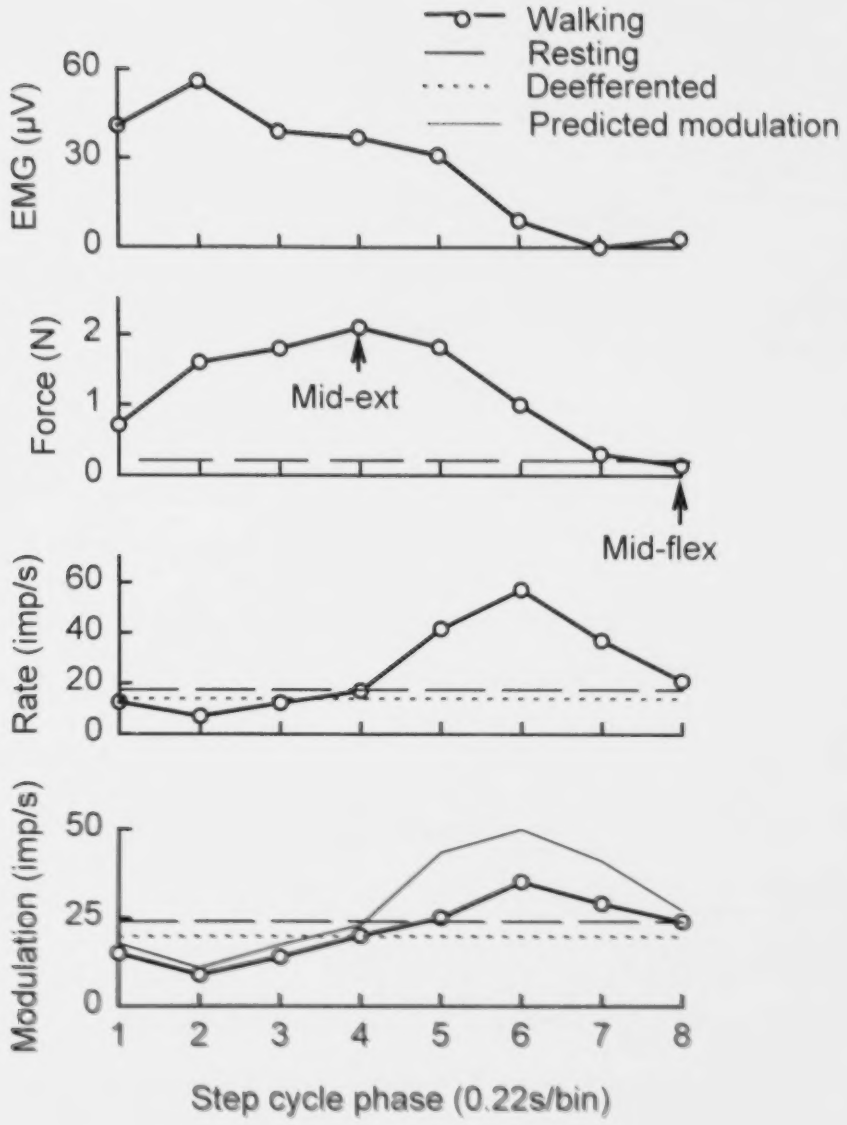

Figure 3.4 - Soleus EMG, force, afferent firing rate and modulation during a step cycle. The firing rates are the means of the PSTH responses computed in Fig. 3.1. Modulations in firing are the amplitudes of the best fit sine waves to the PSTH responses in Fig. 3.1. Dashed and dotted lines show afferent firing in the resting and deefferented states respectively. The thin line in the lower panel indicates the modulation that is predicted from the rate assuming that the unit has no γ-drive (using the deefferented rate-modulation relation of Fig. 3.2*H*). The two arrows indicate times when the rate of change of force (not shown) was zero, and are referred to in the text as mid-extension and mid-flexion. Note that the predicted modulation (thin line) matches the actual modulation, particularly at mid-flexion and mid-extension, indicating that this afferent was likely without γ-drive.

When locomotion commenced the averaged firing rate increased markedly as compared to the resting state (Fig. 3.5; solid symbols), with significant increases both in mid-flexion (open circles) and mid-extension (open squares; $p<0.05$). Also, the sensitivity decreased significantly during both flexion and extension. Both the averaged rate and sensitivity were not significantly different during mid-flexion and mid-extension ($p>0.1$), indicating that a tonic γ-drive was introduced during locomotion. An estimate of the averaged effect of γ-drive during locomotion was calculated by averaging the mean firing rates (and modulations) occurring at mid-flexion and mid-extension. The averaged rate during locomotion increased by 121%, and the sensitivity (modulation) decreased by 32%, both changes were significant ($p<0.05$). Averages under all conditions are summarized in Table 3.1.

For reference, two thick arrows are drawn in Fig. 3.5 that indicate how both modulation and firing rate change with dynamic or static γ-motoneurone activation. The directions of these arrows were taken from Table 3.1 of Taylor *et al.* (1985), summarizing the results from experiments in which they directly stimulated γ-motoneurones and recorded changes in afferent firing rate and sensitivity. The lines through the arrows are drawn starting at the deefferented mean values, so that points on the lines represent responses to either dynamic or static γ-motoneurone activation in isolation. These two lines provide a new set of 'axes' by which to interpret the data (approximately). Clearly the units at rest lie near the γ_d axis, suggesting that they were predominantly activated by dynamic γ-drive. During locomotion the γ-drive was increased in the direction of the γ_s axis, suggesting that a predominantly tonic increase in static γ-drive occurred.

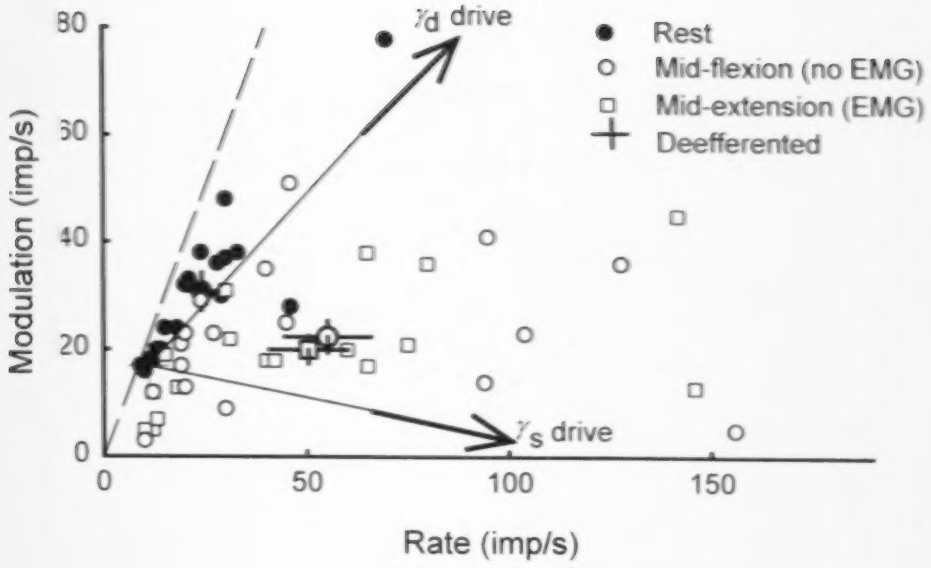

Figure 3.5 - Summary of modulations to stretch and mean firing rates for the 18 soleus afferents investigated in Clonidine-treated spinal cats.
For each unit, results are shown for the resting state and for walking during mid-flexion and mid-extension. Means values for each condition are shown by larger symbols, associated crosses indicate the standard error. Cross near origin indicates the mean response of deefferented units at the standard length. Diagonal lines with thick arrows indicate the effects of either dynamic or static γ-drive in isolation (taken from Taylor *et al.* 1985, scatter not shown but suggested by thick arrows). Dashed line: maximal modulation possible at a given rate (see METHODS). Standard 0.25 mm, 4 Hz stretch used in all cases. Note that a third of the units are clustered in the lower left corner, since they had very low firing rates, with minimal rates during the extensor contraction (square symbol). On average, however, the mean firing rate increased and the modulation decreased during locomotion, indicating a tonic increase in static γ-drive.

Afferents without γ-drive. Primary afferents with averaged firing rates of less than 20 imp/s during locomotion (average of mid-flexion and mid-extension points; see above) were classified as 'undriven' by γ-motoneurones. Though this dividing line is somewhat arbitrary, it corresponds to 2 standard deviations above the mean firing rate of the units after deefferentation (at the standard length, without ramp stretches). These undriven afferents also showed characteristic unloading during contractions as described in Fig. 3.4 above. From the 18 afferents studied, 6 (33%) were undriven by this classification, with a mean firing rate of 5 imp/s above the deefferented mean (a small but significant difference, $p<0.05$). Further, prior to locomotion these 6 afferents had a mean rate that was not significantly greater than the deefferented mean ($p>0.05$), suggesting that they were also without γ-drive in this resting state (see Table 3.2). All of these undriven afferents came from 3 of the 6 Clonidine-treated spinal cats. However, in these 3 cats there were also units with γ-drive. Also note that the locomotion was less vigorous for the cat in Fig. 3.3 with undriven units (see the lower amplitude force and EMG signals in Fig. 3.3*B* compared to 3.3*A*). Table 3.2 compares the averaged firing rates for these undriven afferents to the remaining afferents with γ-drive.

3.3.2 DOPA-treated spinal cats

Since we found that a third of the primary afferents in Clonidine-treated cats had little or no γ-drive, we wondered whether this lack of γ-drive might be due to Clonidine, and not to the spinal locomotor state. Such undriven units have not been reported before during spinal locomotion produced by DOPA (Sjöström & Zangger, 1976). We therefore investigated spinal cats treated with DOPA (and Nialamide) to produce locomotion.

	Undriven units (n=6)		Driven units (n=12)	
Condition	Rate (imp/s)	Modulation (imp/s)	Rate (imp/s)	Modulation (imp/s)
Resting	13±3	20±3	30±16	37±16
Mid-flexion in walking	20±5	21±6	73±45	23±15
Mid-extension in walking	13±3	10±6	69±38	25±10
Mean in walking	16±2	16±4	71±40	24±11
Deefferented	11±5	17±4	-	-

Table 3.2 - Firing behaviour of Ia afferents in Clonidine-treated spinal cats (mean±SD). Same format as Table 3.1.

Resting state: dynamic γ-drive. The DOPA-treated spinal cats produced locomotor activity spontaneously, and therefore there was no equivalent to the 'resting' state seen in Clonidine-treated cats. However, prior to administration of DOPA, locomotion is usually not present, and it is known that the γ-drive is mostly dynamic (Alnæs, Jansen & Rudjord, 1965). In 9 units we verified this result, showing that primary afferents in untreated spinal cats had large rate modulations during the standard test stretch (mean of 46 imp/s) and relatively low firing rates (mean of 42 imp/s), as for the resting Clonidine-treated cats (see Table 3.1).

Walking state: phasic static γ-drive. After the administration of DOPA, locomotion commenced with EMG and force levels similar to that seen in Clonidine-treated cats, although the locomotion was characteristically slower, as shown in Fig. 3.6. As with the Clonidine cats, the onset of locomotion was accompanied by significant overall increases in

the primary afferent firing rates (mean increase: 40%), and decreases in sensitivities (mean decrease: 50%, $p<0.05$, n=10 units, see Table 3.1), suggesting the initiation of static γ-drive.

However, in the DOPA-treated cats there were marked differences in the γ-drive during the flexion and extension phases of locomotion. This phasic variation in γ-drive is clearly seen even in the unaveraged single step cycle shown in Fig. 3.6. Specifically, during early flexion (left of Fig. 3.6) the primary afferent firing was low, indicating little γ-drive. Toward the end of flexion the afferent firing jumped markedly (left arrow), and initially did not respond to the imposed stretch, suggesting that static γ-drive was initiated. This static γ-drive preceded the extensor α-motoneurone activation (EMG onset, right arrow) by 500 ms, and probably lasted throughout the extension phase. After the EMG onset, the afferent sensitivity increased, suggesting that dynamic γ-drive was also present, coactivated with the EMG (also note the increase in firing at the end of extension, due to the high sensitivity and the rapid muscle relengthening as the force dropped). However, the sensitivity during extension was not always as great as in this afferent; on average it was lower than the sensitivity in the resting state (see also Table 3.1).

The results from the 10 primary afferents recorded during locomotion in DOPA-treated cats are summarized in Fig. 3.7, using the same analysis described above for the Clonidine-treated cats (Fig. 3.5). Most afferents (9 out of 10) fired phasically during the step cycle, as already described for Fig. 3.6. During flexion (without EMG) afferent firing rates and sensitivities were significantly lower than in the resting pre-DOPA cat ($p<0.05$), suggesting that the dynamic γ-drive was phasically inhibited. During extension the mean afferent firing rate increased by 112% and sensitivity decreased by 41% when compared to

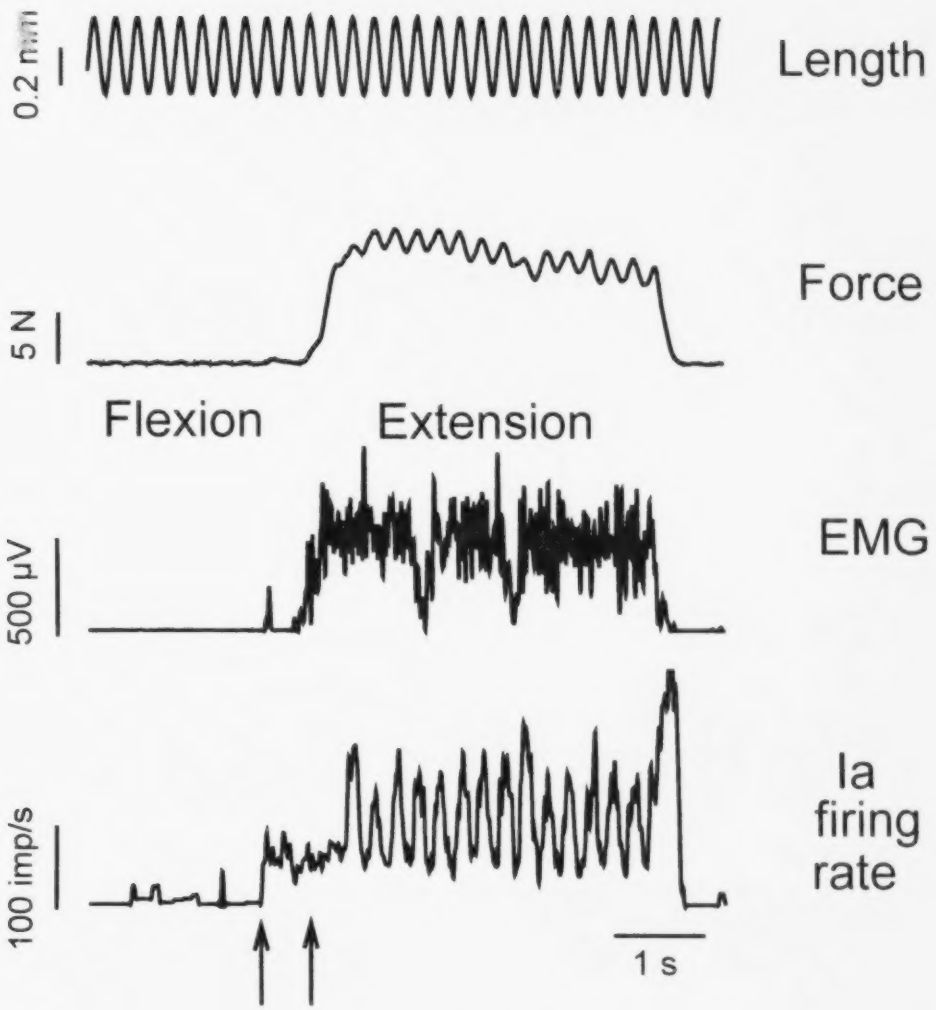

Figure 3.6 - Soleus primary muscle spindle afferent in DOPA-treated spinal cat during locomotion.

Same format as Fig. 3.3. Only one step cycle shown, in order to have the time resolution to see modulations in afferent firing during stretch. Note that 1) the step cycle is considerably longer during DOPA induced walking, compared to that with Clonidine; 2) the afferent firing is phasic; 3) the afferent firing onset (left arrow) precedes the EMG (right arrow) by 500 ms and does not initially respond to the imposed stretch (static γ-drive); and 4) the afferent sensitivity increases after the onset of EMG (coactivated dynamic γ-drive).

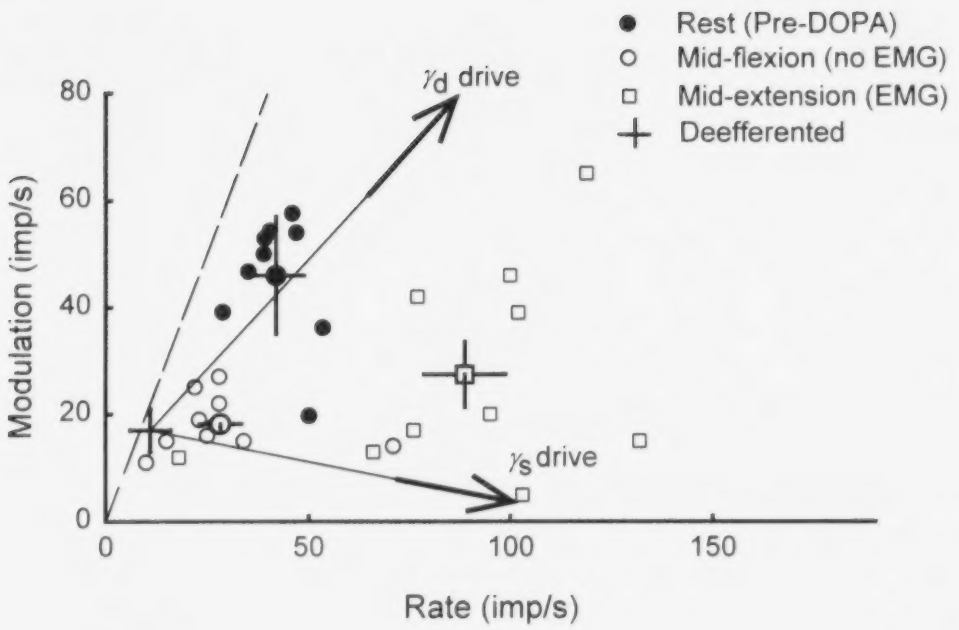

Figure 3.7 - Summary of modulations to stretch and mean firing rates for the 10 soleus afferents investigated in DOPA-treated spinal cats.
Same format as Fig. 3.5. Note that during locomotion the sensitivity dropped when compared to the resting state. During flexion this drop was associated with a decreased firing rate, suggesting a decreased dynamic γ-drive. During extension there was a large increase in firing rate, suggesting additionally an increased static γ-drive.

the resting state, suggesting a static γ-drive, comparable to that in the Clonidine-treated cats. This γ-drive preceded the EMG by 100-500 ms. Only one afferent had an overall mean rate during walking of less than 20 imp/s, classifying it as an undriven unit. Thus, undriven units were likely related to the use of Clonidine. Further support for this conclusion is provided by the use of Clonidine in decerebrate cats as described below.

The phase-dependent γ-drive in DOPA- and Clonidine-treated cats can be estimated for the individual units by subtracting the afferent firing rates (and modulations) during flexion from those during extension. In DOPA cats, 90% of afferents increased their firing rate during extensor activity, all by more than 20 imp/s (mean increase: 67 imp/s). In contrast, in Clonidine cats, no unit increased its rate phasically by more than 20 imp/s. The difference between the two preparations can be clearly seen when comparing the mean firing rate for each phase on Figs. 3.5 and 3.7. In the DOPA cats, the mean firing rate of the units is much greater in mid-extension (large open square) than in mid-flexion (large open circle). In the Clonidine-treated cats, those means are almost identical (not significantly different; see above). Also, in the DOPA cats, the change in sensitivities between the two phases was variable with a mean increase of 9 imp/s during mid-extension.

3.3.3 Other afferents during walking

Group II afferents. In the DOPA-treated cats 4 secondary muscle spindle afferents were studied during locomotion with a 4 Hz, 1 mm test oscillation (larger amplitude needed as they were less sensitive to stretch). These units had a mean firing rate in the resting state of 48±17 imp/s, which increased significantly ($p<0.05$) during both flexion and extension to 69±24 and 103±37 imp/s respectively (overall mean increase during walking was 79%). Since

group II afferents predominantly innervate chain and static nuclear bag_2 fibres (Gladden,1992), these rate changes can be interpreted as static γ-drive, consistent with the above observations on Ia afferents. The modulation to stretch in these units also dropped significantly ($p<0.05$) during walking; mean values during resting, flexion and extension were respectively: 27±5, 13±3 and 15±5 imp/s (mean decrease during walking was 48%).

Group Ib afferents. Since one objective of the present study was to correlate afferent sensitivity changes to changes in reflex responses during locomotion (Akazawa *et al.* 1982; see also Chapter 5), we also analyzed five Ib afferents. Prior to locomotion and during the flexion phase, when there was no active force production, these afferents were often silent or fired at low rates (mean: 12 imp/s). However, during the extension phase they increased their firing rates along with the force. At mid-extension the mean force was 9±3 N (±SD) and the afferent firing rate was 107±41 imp/s. During the standard 4 Hz, 0.25 mm sinusoidal stretch the firing rate modulation also increased with the force, likely because the stiffness increased with the force (Hoffer & Andreassen, 1981). At mid-extension the rate modulation was 28±7 imp/s. Thus, during extension the sensitivity of these Ib afferents was comparable to that of Ia afferents (e.g. Fig. 3.7), which indicates that they could, at least in principle, participate significantly in the stretch reflex (Gossard & Hultborn, 1991; see also Chapters 4 and 5). However, Ib units responded more slowly to the 4 Hz stretch than Ia units, with a phase lead of only 30-50^0, compared to a phase lead of 80-100^0 in Ia afferents (Fig. 3.1), probably due to slower receptor dynamics (Houk & Simon, 1967; Chen & Poppele, 1978).

3.3.4 Decerebrate cats

Group Ia afferents. Since the stretch reflex of ankle extensors in decerebrate cats decreases with tonic contractions produced either spontaneously (Akazawa *et al.* 1982) or by crossed extensor reflex activation (Chapters 4 and 5), we have investigated whether this reduction is in part due to decreased spindle sensitivity, as appears to be the case during locomotion. Fig. 3.8 shows an example of the firing of a primary afferent before and after the crossed extensor reflex activation (contralateral CP nerve). In the 'resting' state, before stimulation, the afferent was very sensitive to stretch, but had little offset in firing rate, as for the resting spinal state. Upon stimulation a contraction developed and the afferent appeared to receive static γ-drive, judging from its increase in firing rate and decrease in sensitivity.

Fig. 3.9 summarizes the results from the 18 afferents studied in the decerebrate cats, analyzed in the same manner as for the spinal cats in Figs. 3.5 and 3.7 (10 of these afferents were held until after spinalization, and thus were also included in the spinal Clonidine locomotion studies). Prior to crossed extensor reflex activation, the mean firing rate was relatively low (31±11 imp/s; though significantly above the deefferented mean rate, $p<0.05$) and the modulation to stretch was high (38±11 imp/s), suggesting dynamic γ-drive. See Table 3.1, and the large solid circle indicating the means in Fig. 3.9.

During an abrupt onset of the contralateral CP nerve stimulation, the increased afferent firing usually started with the ipsilateral soleus EMG, as shown in Fig. 3.8. However, more gradual increases in stimuli produced increases in afferent firing without changes in EMG (not shown). Thus, in Fig. 3.9 two values are shown for each unit, one without EMG changes (open circles), and one with EMG changes (open squares), analogous to the results obtained during locomotion in flexion and extension respectively. During crossed extensor

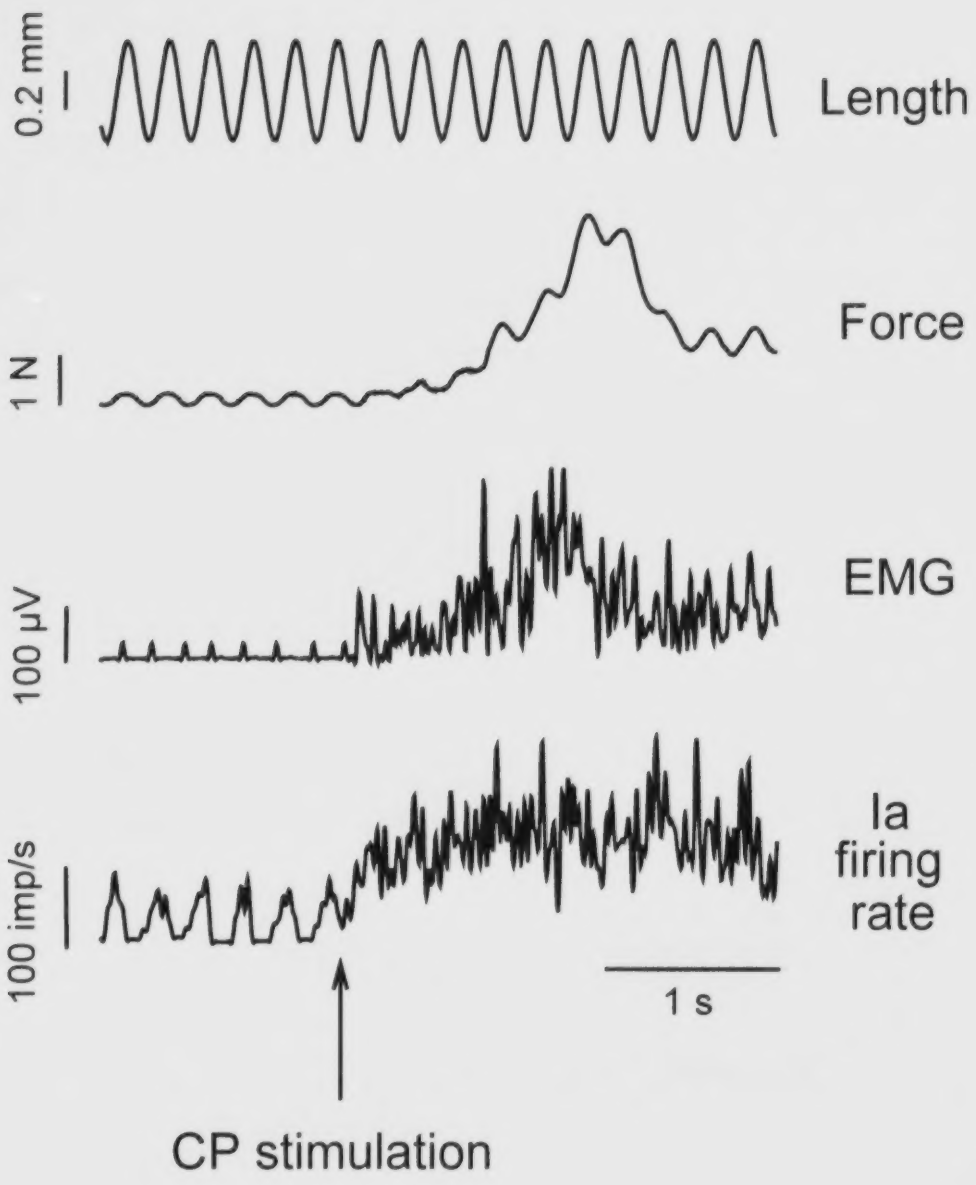

Figure 3.8 - Soleus primary afferent firing during crossed extensor reflex activation in a decerebrate cat.
Same format as Fig. 3.3. Note the stretch-related modulation in firing before the onset of crossed extensor reflex activation (arrow), which gives way to strong unmodulated firing afterwards.

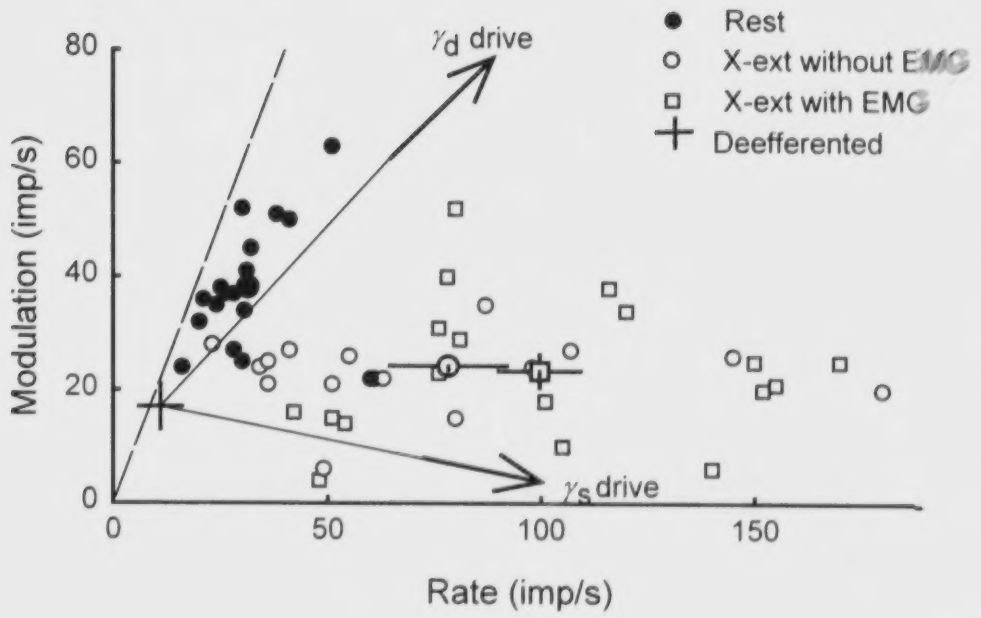

Figure 3.9 - Summary of modulations to stretch and mean firing rates for the 18 soleus primary afferents investigated in decerebrate cats during crossed extensor reflexes (X-ext). Same format as Fig. 3.5. Firing shown in the resting state before crossed extensor reflex activation, and during CP stimulation without and with accompanying soleus EMG. Note the tonic increase in rate and decrease in sensitivity during crossed extensor activity.

reflex activation the mean firing rate increased significantly ($p<0.05$) over that in the resting state, both with and without EMG, compare large open circle and square to large filled circle in Fig. 3.9 (mean increase of 187%). The firing rates with and without EMG were not significantly different ($p>0.05$). Despite this large rate increase, the sensitivity decreased significantly, with a mean drop of 37% (see Table 3.1). Based on the γ_s and γ_d reference lines from the data from Taylor et al. (1985, described above), it appears that a predominantly static γ-drive was introduced during the crossed extensor reflex, as in locomotion. The dynamic γ-drive changed little in comparison.

Group II afferents. To verify the conclusion of increased static γ-drive during crossed extensor reflex activation we were able to examine 4 secondary muscle spindle afferents in the decerebrate cats. These units had a mean firing rate in the resting state of 16±3 imp/s, which increased significantly ($p<0.05$) with crossed extensor reflex activation without and with EMG responses to 37±5 and 54±22 imp/s respectively (mean increase of 180%). The corresponding modulations to a 4 Hz, 1 mm stretch dropped during crossed extensor reflex activation (by 13%, though not significantly, $p>0.1$), from 19±6 imp/s in resting state to 16±5 and 17±5 imp/s without and with EMG respectively.

Effect of Clonidine. Unlike in Clonidine-treated spinal cats, there were no afferents in decerebrate cats that unloaded during contractions (undriven afferents, compare Figs. 3.5 and 3.9). To more directly test the effect of Naloxone and Clonidine we administered these drugs while following seven Ia afferents with strong γ-drive in the decerebrate state. Prior to drug administration the mean firing rates during resting and tonic contractions (with EMG) were respectively 31±13 and 53±27 imp/s, similar to the overall means in Table 3.1. With the

administration of Naloxone no significant changes occurred in the firing of these units. However, 15 minutes after the administration of Clonidine the firing of all units decreased markedly, in the resting state (by 29%) and during contractions (by 79%), as shown in Table 3.1 (significant at $p<0.05$). During contractions three units stopped firing altogether, as shown in Fig. 3.10. All units decreased their firing rates and had mean firing rates that were not significantly different from the deefferented means. They were all classified as 'undriven' since their rates were less than the 20 imp/s cut-off rate, as described for the spinal cats above. The sensitivities also dropped significantly with Clonidine, as seen in Table 3.1. One group II unit was also tested before and after Clonidine. Its firing rate and sensitivity were not affected by Clonidine.

The effects of Clonidine on afferent firing were probably mediated centrally at the γ-motoneurones. However, as Clonidine is a strong hypotensive drug, it could influence the spindles peripherally by producing ischaemia. This was probably not happening, since the blood pressure did not drop markedly (though the heart rate slowed) during Clonidine administration in the 3 decerebrate cats described above. Furthermore, Clonidine had no effect on muscle spindle firing when given after cutting the ventral roots (one cat tested, five afferents).

3.4 DISCUSSION

We have shown that when locomotion commences in spinal cats the firing rate of ankle extensor muscle spindle afferents increases, and the sensitivity (modulation to stretch)

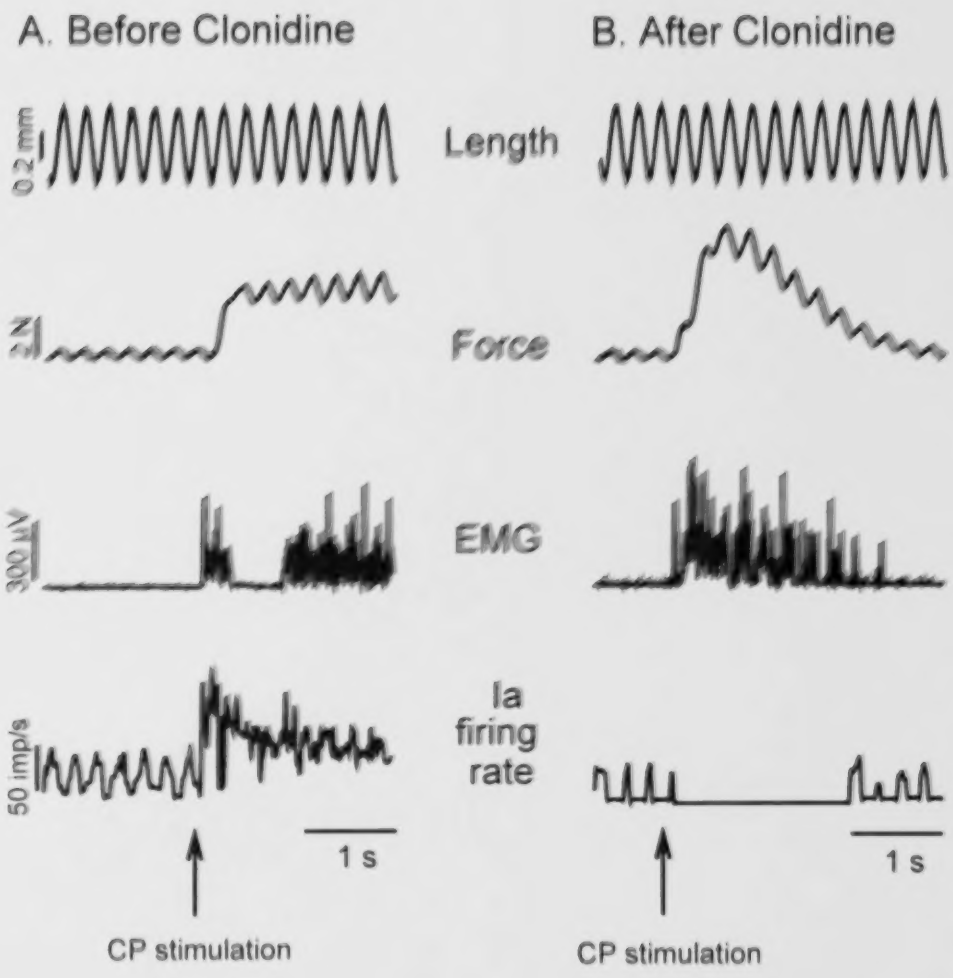

Figure 3.10 - Soleus primary afferent firing in a decerebrate cat before and after Clonidine.

A. The response of a unit to stimulation of the common peroneal nerve to elicit a crossed extensor reflex, as in Fig. 3.8. *B*. The response of the same unit after administration of Clonidine. Note that rather than increasing its firing rate, the discharge ceased, indicating a profound inhibition of γ-drive by Clonidine.

decreases, as in decerebrate locomoting cats (Taylor *et al.* 1985). Such changes also occurred during the crossed extensor reflex in decerebrate cats, extending the results from phasic (Taylor *et al.* 1985) to tonic contractions (see also Jansen & Rudjord, 1965). These changes in afferent firing can be unambiguously interpreted as being due to increased static γ-drive, since the muscle was held at a preset length, and afferent firing was analyzed at times when internal lengthening due to contractions was minimal. Additionally, we have shown that prior to locomotion the afferent firing rate and sensitivity were higher than in the decerebrated state, consistent with the effects of dynamic γ-motoneurone activity (Alnæs *et al.* 1965, Taylor *et al.* 1985). There are, however, a number of differences between various preparations, as summarized in Table 3.3.

3.4.1 Comparison to γ-motoneurone stimulation studies

The above description of the changes in γ-drive can be made more precise by comparison to afferent firing patterns during direct γ-motoneurone stimulation (Taylor *et al.* 1985). If the effects of dynamic and static γ-drive on afferent firing sum linearly, we can compute the effective dynamic and static γ stimulation needed to produce the change in firing rate and sensitivity seen when locomotion commenced (using the rate and sensitivity responses to γ stimulation shown in Table 3.1 of Taylor *et al.* 1985).

	Dynamic γ		Static γ	
Rest	↑↑		0	
Tonic contractions	Change in γ from rest		Change in γ from rest	
Crossed extensor	0		↑↑	
Crossed ext. with Clonidine	↓		0	
Walking preparations	Change from rest	Modulation in step cycle	Change from rest	Modulation in step cycle
Decerebrate walking	↓	↑↑	↑↑	↑
Spinal with DOPA	↓	↑	↑↑	↑
Spinal with Clonidine	↓	0	↑	0
Chronic (intact)	0	0	↑↑	0

Table 3.3 - Summary of γ-motoneurone activity in different cat preparations.
Symbols: '↑' = high activity (rest) or increase in activity; '↓' = decrease in activity; '0' = little measurable activity (rest) or no significant change. In walking preparations 'change from rest' is the mean change in γ for the whole step cycle. Sources: Resting state, Alnæs *et al.* 1965 and present study; Tonic contractions in premammillary decerebrate cats, present study; Premammillary decerebrate walking cats, Taylor *et al.* 1985; Spinal DOPA cats, Grillner *et al.* 1967 and present study; Clonidine cats, present study; Chronic cats during slow walking, Prochazka *et al.* 1988. Results mostly refer to hindlimb extensor γ-motoneurones.

For example, to produce the changes in firing seen in the mean afferent in Fig. 3.5, a dynamic γ-motoneurone would need to be stimulated with 33 imp/s *less* than in the resting state and a static γ-motoneurone with 100 imp/s *more*. This is only a first approximation, since the effects do not sum linearly and spindles are presumably influenced by more than one dynamic and one static γ efferents (e.g. Matthews, 1972). Similar conclusions are reached

from calculations of the effective change in γ-drive during walking in DOPA cats and during crossed extensor reflex activation in decerebrate cats. Additionally, in the DOPA cats such calculations show that although during locomotion the averaged dynamic γ-drive is decreased (and static drive increased) compared to the resting state, it is phasically increased during the extensor phase of locomotion (Fig. 3.7) as in the decerebrate walking preparation of Murphy *et al.* (1984).

3.4.2 Independence of static γ and α-motoneurones

The static γ-drive during movement is relatively independent of the α-motoneurone activity. It may be present for none, part or all of the locomotor cycle. Also, in the decerebrate cats during crossed extensor reflexes the γ-drive can be increased before the contraction starts. These results are consistent with the well known notion that static γ-motoneurones are often recruited before α-motoneurones during movements in cats (e.g. Loeb, Hoffer & Pratt, 1985; Bessou *et al.* 1986; see also description of 'fusimotor windup' in Prochazka *et al.* 1985). This normal recruitment of static γ-motoneurones before α-motoneurones is not fixed however, since we have shown that it can be completely reversed with Clonidine for certain units.

The current results indicate that γ-drive to certain spindles can be eliminated without eliminating α-motoneurone activation. This α without γ-drive is not specific to locomotion, occurring also in decerebrate and resting spinal cats. In spinal cats with such 'undriven' spindles there were also units with γ-drive, which raises the possibility that Clonidine has

selective action on a sub-population of the γ-motoneurone pool. Clonidine acted more uniformly on the whole γ-motoneurone pool in decerebrate cats (reducing drive in 7 out of 8 units).

Clonidine has been used in humans as an antispastic agent (Nance, Shears & Nance, 1985; Weingarden & Belen, 1992), though the mechanism of action has been unknown. Its profound effect on the fusimotor (γ) system could account for much of this action. Clonidine is also well known as a hypotensive drug (Gilman, Goodman & Gilman, 1980). As mentioned in the RESULTS, the peripheral hypotensive action of Clonidine was unlikely to have played a role by directly reducing muscle spindle sensitivity by inducing ischaemia, but we cannot completely rule out such effects.

3.4.3 Spinal origin of static γ-drive

Taken together, the results from the different spinal, decerebrate and intact cat preparations suggest that a strong static γ-drive is normally introduced in preparation for and during locomotor and non-locomotor movements, both in extensor and flexor muscles (Murphy *et al.* 1984; Loeb *et al.* 1985; Taylor *et al.* 1985; Prochazka *et al.* 1985; Bessou *et al.* 1986; Prochazka, Hulliger, Trend & Dürmüller, 1988; Hulliger *et al.* 1989; see also Table 3.3). The similarity of the static γ-drive seen in all locomoting preparations, including the spinal cat, suggests that a common *spinal* circuit is responsible. Further, the static γ-drive seen during the crossed extensor reflex in the decerebrate cats could be a manifestation of the same spinal circuitry. As a crossed extensor reflex activation (i.e. FRA) is thought to elicit a step from the spinal rhythm generator in DOPA-treated spinal cats (Jankowska *et al.* 1967;

see also INTRODUCTION), the same stimulation might elicit a prolonged 'step' in the decerebrate cat (with associated static γ-drive, Grillner *et al.* 1967). This is especially likely since the decerebrate cat is known to have a strong descending monoaminergic activation, replaced by DOPA in the spinal cat (Jankowska *et al.* 1967).

There are differing degrees of phasic activation of this static γ-drive with the changing α-motoneurone activity during locomotion, even in the spinal cat (Figs. 3.3 and 3.6). However, this may, in part, be explained by differences in speed of locomotion, rather than fundamentally different control mechanisms. Specifically, the lower recruitment threshold for static γ-motoneurones causes them to come on before and outlast the α-motoneurone activity during locomotion (by 100-500 ms; see also Bessou *et al.* 1986). Thus, when locomotion is fast (1 s step duration; e.g. Clonidine-treated spinal cats; Fig. 3.3*A*), the γ-drive from one step cycle may overlap with that from the next step, producing effectively tonic γ-drive. A linkage of the γ and α-motoneurone activation is only clearly apparent in slower locomotion (e.g. in DOPA cats: extensor muscles, Fig. 3.6; flexor muscles, Grillner *et al.* 1967, Sjöström & Zangger, 1976), and even then the γ-drive outlasts the α activity.

It is also evident from this discussion that the central drive to the γ-motoneurones is *not* reciprocally organized between flexors and extensors, as for α-motoneurones. In the decerebrate cat, for example, the static drive in extensors is strongly activated throughout the step cycle (Murphy *et al.* 1984, Bessou *et al.* 1986), even though there is also strong static γ-drive in the flexors (Murphy & Hammond, 1993; Cabelguen, 1981). Similar conclusions likely hold for Clonidine-treated spinal cats (Fig. 3.3*A*), although evidence for static γ-drive in flexors is only available for DOPA-treated cats (Sjöström & Zangger, 1976).

3.4.4 Functional implications

Prior to movement the predominantly dynamic γ-drive serves a postural role, as it augments the spindle sensitivity, and thus strengthens the reflex responses to small disturbances mediated by Ia afferents (e.g. Akazawa *et al.* 1982; see also Chapters 4 and 5). The lack of presynaptic inhibition on Ia afferents (pre-DOPA/resting spinal cat: Andén, Jukes, Lundberg & Vyklicky, 1966; standing human: Capaday & Stein, 1986) and the high short range stiffness of muscle (Rack & Westbury, 1969) at this time further augment such reflex maintenance of posture.

With the initiation of movement, the increased static γ-drive enables group Ia and II afferents to usefully signal muscle length and velocity, in spite of rapid shortening contractions. Further, this increased static γ-drive decreases the spindle sensitivity and thus weakens the reflex responses measured during movement (Chapters 4 and 5). The minimal dynamic γ-drive and increased presynaptic inhibition also associated with locomotor movements further decreases the action of the stretch reflex (Andén *et al.* 1966: during DOPA induced locomotor-like FRA responses; Capaday & Stein, 1986: during walking in humans). Together these effects assure that the stretch reflexes do not resist the movement, or produce instability. Dynamic γ-drive appears to be augmented during movements only at times when the stretch reflex is useful, such as in the extensor muscles during stance (Figs. 3.6 and 3.7; see also Taylor *et al.* 1985). Additionally, during extensor contractions the extensor group Ib afferents can be as sensitive as the Ia afferents (see RESULTS), and may further augment stretch responses via polysynaptic pathways (Conway, Hultborn & Kiehn, 1987).

These changes in afferent control reflect the differing role that afferents play during locomotion, compared to during postural tasks. Rather than being used simply in a traditional servo-assistive mode, afferents are used to signal the state of the limb and to trigger or reset the timing of the centrally generated locomotor rhythm (e.g. Grillner & Rossignol, 1978; Conway *et al.* 1987; see reviews by Prochazka, 1989 and Gossard & Hultborn, 1991). Whatever the details of the role of afferents in locomotion, it is clear from the present study that there are common features in the control of γ-motoneurones during locomotion in all preparations (e.g. static γ-drive inferred from afferent firing behaviour; Table 3.3), presumably related to the requirements of weight support and signalling of muscle length during rapid movements. However, there is considerable flexibility in the γ control, allowing tailoring of the spindle sensitivity to task requirements ('fusimotor set': Prochazka, 1989). Surprisingly, we have found that it is even possible to activate α-motoneurones without significantly recruiting γ-motoneurones under some conditions (e.g. Clonidine-treated decerebrate cats during crossed extensor reflexes).

Post Scriptum

This chapter has been accepted for publication:

BENNETT, D.J., DE SERRES, S.J. & STEIN, R.B.
Regulation of soleus muscle spindle sensitivity in decerebrate and spinal cats during postural and locomotor activities.
***Journal of Physiology*, (in press)**

I completed the realization of this project, after Dr. Bennett left Edmonton. I was therefore involved in data acquisition and analysis for 4 of the 15 cats, and I participated in the preparation of the manuscript.

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CHAPTER 4

GAIN OF THE TRICEPS SURAE STRETCH REFLEX IN DECEREBRATE AND SPINAL CATS DURING POSTURAL AND LOCOMOTOR ACTIVITIES

4.1 INTRODUCTION

With the initiation of locomotor activity in cats, primary muscle spindle (Ia) afferent sensitivity is reduced due to an increased static γ-motoneurone activity (Bergmans & Grillner, 1968; Taylor, Stein & Murphy, 1985; Prochazka, 1989; see also CHAPTER 3). Also, Ia afferent transmission to motoneurones is inhibited, likely by presynaptic inhibition, both in cats (Bayev & Kostyuk, 1982; Gossard, Cabelguen & Rossignol, 1991) and humans (Capaday & Stein, 1986; Yang & Whelan, 1993). In contrast, excitatory polysynaptic pathways are opened during locomotion (from Ib afferents: Conway, Hultborn & Kiehn, 1987; Pearson, Ramirez & Jiang, 1992; Gossard & Hultborn, 1991; and group II afferents: Perreault, Angel, Guertin & McCrea, 1995). Despite understanding of these various reflex components, the question of how much afferent feedback contributes to motoneurone output during locomotion remains largely unanswered. Yang, Stein & James (1991) calculated that in human locomotion the stretch reflex contributes 30-60% of the EMG in soleus muscle during much of the stance phase. Here, we have re-investigated this question in cats by quantifying the strength or 'gain' of

afferent feedback throughout the locomotor cycle, and comparing it to the gain prior to locomotion and during tonic contractions.

The mammalian stretch reflex is difficult to quantify because the sensors (muscle spindles and tendon organs) are an integral part of the muscle; one cannot be stretched without the other, as would be necessary to measure the 'open loop' reflex gain. For this reason conflicting uses of the term reflex 'gain' have arisen in the literature, which generally only define part of the reflex loop. In simpler systems, such as stick insects, where the sense organs are separate from the muscle, the open loop gain has been determined by oscillating (stretching) the sense organ and measuring the relative *magnitude and phase* of the resulting muscle/limb movements (a unitless input/output ratio; Bässler, 1983).

Recently the open loop gain (or gain for short) has been estimated in humans by a two-part experiment, mimicking Bässler's stick insect experiments (with the elbow joint, Bennett, Gorassini & Prochazka, 1994). During posture the gain magnitude is often near 1, indicating that the afferent activity following a 1 mm stretch of the muscle receptors should cause a 1 mm muscle shortening under isotonic conditions. This gain is sufficiently high that tremor would occur if it were increased further (Bennett *et al.* 1994; see also Allum *et al.* 1982; Carter, Crago & Keith 1990). During voluntary movements in humans the reflex gain is much lower, but increases rapidly (to about 1) near the end of the movement, where the reflexes likely function to arrest the limb at the desired target (Bennett, 1994). Numerous results from cat experiments comparing stretch responses before and after eliminating afferent feedback (e.g. by deafferentation) indicate that the reflex gain is also high during tonic contractions in cats (Houk, Singer & Goldman, 1970; Rosenthal, McKean, Roberts & Terzuolo, 1970; Goodwin, Hoffman & Luschei, 1978; Hoffer & Andreassen, 1981). No

comparable deafferentation studies have been performed during locomotion. Akazawa, Aldridge, Steeves and Stein (1982) used rapid pulsatile stretches to evaluate the reflex responses during locomotion in decerebrate cats and found the reflexes to be highly modulated with the step cycle. However, such rapid stretches do not give an indication of the reflex effects during slower muscle length changes, such as occur during much of the locomotor cycle.

We have thus estimated the reflex gain of cat hindlimb muscles during locomotion, using sinusoidal stretches applied to the triceps surae muscles in a spinal preparation. The stretch frequency was varied to cover the frequency ranges of voluntary movement and tremor. The test muscle was held at a preset length and stretched in a controlled manner without disturbing the rhythm ('isometric locomotion'; Pearson *et al.* 1992), thus minimizing length, velocity, and amplitude dependent nonlinearities of the stretch reflex (e.g. Stein & Kearney 1995). The spinal locomoting cat preparation was chosen for reasons including: (1) relative ease of producing locomotion pharmacologically (with Clonidine), (2) simplicity of interpretation, in that any reflex modulation can be ascribed to spinal mechanisms (see CHAPTER 3), and (3) clinical relevance to the recovery of locomotion and reflex function in spinal cord injured patients (particularly as it relates to Clonidine, an antispastic drug). We show that the reflex gain is markedly lower during locomotion, compared to other 'postural' states.

4.2 METHODS

4.2.1 General preparation

Experiments were performed to determine the reflex gain of the triceps surae (TS) muscles of the left hindlimb of 11 male cats (2.5-4.5 kg), usually in three states: (1) after a premammillary decerebration, (2) after an additional T12 spinalization and treatment with Clonidine to produce locomotion, and (3) after deafferentation by sectioning the ipsilateral L5-S2 dorsal roots (see METHODS in Akazawa *et al.* 1982; Pearson *et al.* 1992). Due to the difficulty in maintaining cats after Clonidine administration, only 5 cats were kept through all 3 states. The remaining cats were only studied in two states: spinal and deafferented (1 cat); decerebrate and deafferented (1 cat); decerebrate and spinal (4 cats). The latter 4 cats were not useful for gain measurements, but the data were used to confirm the force and electromyographic (EMG) results from other cats before deafferentation. The general surgical procedures have been described previously (see METHODS in CHAPTER 3) and are only described here briefly. Under general anaesthesia (Halothane 1.5-2.5% mixed with 95% O_2, 5% CO_2) hindlimb nerves and muscles were prepared as described below and two laminectomies were performed, at T12 and L5-7, in preparation for later spinalization and deafferentation. The cat was then placed in a stereotaxic apparatus and a premammillary decerebration performed (cut precollicular at 45^0), after which the anaesthetic was removed.

4.2.2 Nerve and muscle preparation

Both hindlimbs were extensively denervated (obturator, femoral and hamstring nerves, and all nerves distal to the hamstring nerve.), sparing only the nerves to the TS muscles. A

nerve cuff for TS stimulation was placed on the ipsilateral tibial nerve just behind the knee. On the contralateral leg a cuff was placed on the common peroneal (CP) nerve. The ipsilateral TS muscles were freed from surrounding tissue and the end of the calcaneum bone was cut off, leaving the tendinous insertion of the TS intact. A heavy inelastic thread (#5 silk) was tied either through a hole drilled in the bone chip or around the tendon proximal to the bone. The cat was mounted in a stereotaxic frame and the ipsilateral leg was held slightly extended with a foot clamp and a bone screw inserted in the femur just above the (denervated) knee. The contralateral leg was allowed to swing freely, facilitating locomotion. The TS tendon thread was tied to a muscle puller. The initial muscle length was set by slowly lengthening the muscle during repeated maximal twitch contractions until the maximal twitch force was reached. This length corresponded to an ankle dorsiflexion of about 135^0, and was chosen since it was at the peak of the length-tension curve (Rack & Westbury, 1969), where muscle stiffness changes minimally with small stretches.

4.2.3 Drugs and manual stimulation for spinal locomotion

Following T12 spinalization, Naloxone (25 mg/kg; Sigma Chemical Co., St.Louis, MO, USA) and Clonidine (50-200 mg/kg; Sigma Chemical Co.) were administered to facilitate the neural circuitry involved in locomotion (Pearson *et al.* 1992). Since Clonidine does not produce locomotion by itself, manual stimulation of the skin of the perineal region (rolling pinching motions) was also needed to trigger locomotion. Locomotor rhythmicity was assessed by looking for reciprocal ipsi- and contralateral TS EMG activity, as shown in Fig. 4.1*A*. Rhythmicity was usually not entrained with the rhythm of the manual skin stimulation (Pearson *et al.* 1992), and proceeded at 0.5-1 Hz for periods of 1-2 minutes.

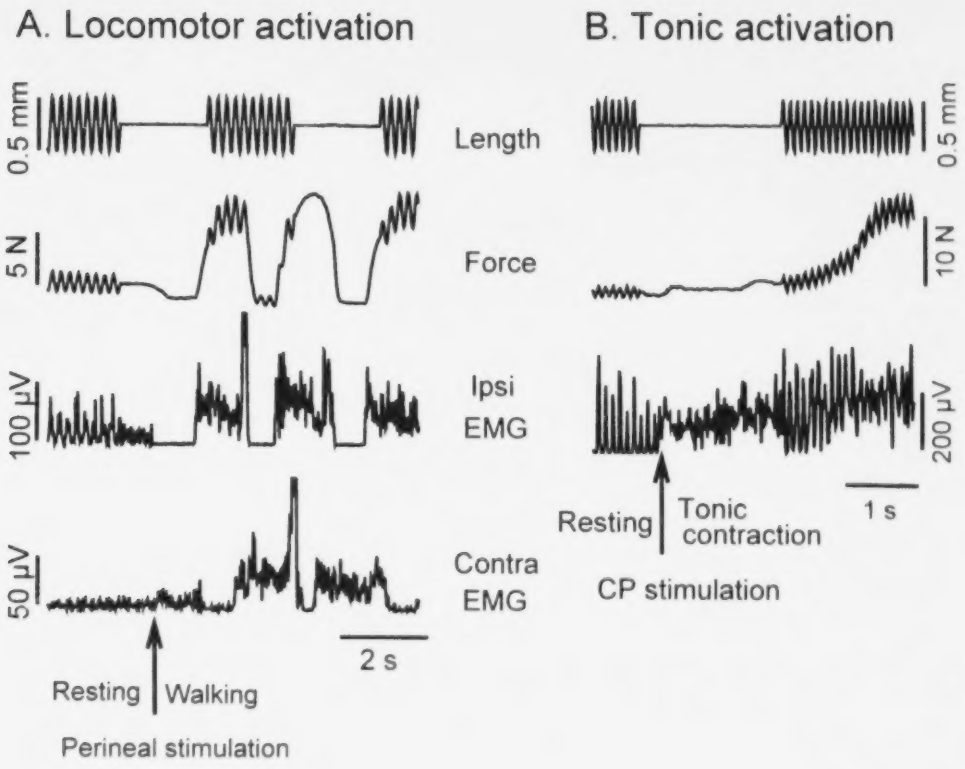

Figure 4.1 - Reflexes during locomotion and tonic contractions.
A. Stretch reflex responses before and after the onset of locomotion in a spinal cat. From top to bottom the traces are respectively: TS length, force, ipsilateral TS EMG (Ipsi) and contralateral TS EMG (Contra). Stretch frequency: 5 Hz. Note the alternating rhythmic EMG activity during manual perineal stimulation, right of the vertical arrow. Also note that the large stretch evoked modulation of EMG seen in the resting state is not present during locomotion. *B*. Stretch reflex responses before and during tonic contractions produced by contralateral CP nerve stimulation (vertical arrow marks onset of stimulation) in a decerebrate cat. Only the beginning of the tonic contraction is shown; the force remained high (>10 N) for a further 6 s. Same format as in part *A*, but contralateral EMG is not shown. Stretch frequency: 10 Hz. Note the large EMG modulation to stretch before the contraction (resting state), which decreased with high level contractions (far right).

4.2.4 Nerve stimulation for tonic muscle contractions

Slowly varying tonic contractions of the TS muscles were produced in the decerebrate state with contralateral CP nerve stimulation (pulses of 0.2 ms at 50 Hz with amplitude graded to generate different contraction levels, see Fig. 4.1*B*). Following spinalization, only phasic (locomotor) contractions could be elicited. Finally, after ipsilateral deafferentation, contractions were produced by perineal stimulation and/or contralateral CP nerve stimulation, whatever produced the best match to contraction levels measured prior to deafferentation. When these methods of producing contractions failed, direct activation of the TS was generated by stimulation of the tibial nerve (pulses of 0.1 ms at 50 Hz). The stiffness/force relationship obtained with this method, in only a few experiments, was probably similar to the relationships measured with either the crossed extension reflex or with manual perineal stimulation (Stein & Gordon, 1986).

During contractions a muscle puller (310B, Cambridge Technology Inc., Watertown, MA, USA) sinusoidally stretched the ipsilateral TS at a fixed frequency with an amplitude of approximately 0.5 mm. No compensation was made on the mechanical stretch size at higher levels of background force, even though a proportion of the stretch may be taken by the muscle tendon (Hoffer, Caputi, Pose & Griffiths, 1989; Elek, Prochazka, Hulliger & Vincent, 1990), therefore decreasing the actual internal length variations seen by the muscle spindle endings. The stretch frequency was adjusted between 1 and 20 Hz in successive trials in the following order: 2, 5, 10, 20, 14, 7, 3, and 1 Hz. However, since the locomotor cycles were on the order of 0.5-1 Hz and very long periods would be needed to separate the stretch evoked activity from the locomotor activity at these frequencies, data are only presented for frequencies higher than 2 Hz.

4.2.5 Signal recording and averaging

Muscle length and force were measured by sensors built into the motor arm. EMG activity was recorded from both TS muscles with two pairs of fine stainless steel wires (Cooner Wire, Chatsworth, CA, USA) inserted with hypodermic needles. For each TS, one wire was inserted in the soleus and the other in the medial gastrocnemius muscle. EMG signals were amplified, highpass filtered at 10 Hz, rectified and low pass filtered at 100 Hz. The amplified length and force signals, as well as the two EMG signals were recorded on tape and digitized off-line with an Axotape system (Axon Instruments Inc., Foster City, CA, USA).

During the slowly varying tonic contractions 8 equal ranges (bins) of force were defined between the minimum and maximum levels of force. For each cycle of the sinusoidal oscillation the mean force level was computed and the stretch response was assigned to the force bin that spanned this mean force. Force and EMG stretch responses in each bin were averaged together.

During locomotion each step cycle was divided into 8 equal periods (phases) in time. All stretch responses (force or EMG) occurring in a given phase were averaged together (see for example, Fig. 4.5 described in RESULTS). The averaged stretch responses were often corrupted by fluctuations in the background contraction (i.e. locomotor related force), particularly at low stretch frequencies. This resulted in a 'trend' in the average not related to the stretch response. The trend for each bin of the step cycle was computed by producing an average uncorrelated with the stretch (i.e. segments of data were taken for averaging at times uncorrelated with the stretch). This trend was then subtracted from the final average to isolate the response related to the stretch. This method of trend removal was verified by repeating it on the length signal which was, of course, without a trend. The responses during locomotion

could also be averaged on the basis of force levels (as done for the slowly ranging tonic contractions), rather than time. During irregular locomotion, this procedure was used and there were no substantial differences in the results from the two methods.

After trend removal, the averaged force and EMG responses, were optimally fitted with sinusoids (by the linear least squares method; Norton, 1986). The remaining non-sinusoidal component (usually small) was estimated by allowing offset and linear components in the fit. The parameters a, b, c, d in the relation:

$$X=a*\sin(wt)+b*\cos(wt)+ct+d$$

were determined, where X is either force or EMG. The magnitude M (where $M^2=a^2+b^2$) and phase θ (where $\tan\theta=a/b$) were thus obtained for each force or EMG response to stretch. Each of these responses can be expressed as a single complex number:

$$R=M*e^{(i\theta)}$$

where $i=\sqrt{-1}$. The ratio of two such complex numbers gives the magnitude and phase shift relating one sinusoidal response to the other:

$$R_1/R_2=M_1/M_2*e^{(i(\theta 1-\theta 2))}$$

This way, the phase of the imposed stretch can be subtracted from all phase estimates to give a value of phase lead relative to the stretch. Similarly, the muscle stiffness (K) can be obtained by dividing the force magnitude by the stretch amplitude:

$$K=F/L=M_F/M_L*e^{(i(\theta F-\theta L))}$$

where muscle compliance=1/K.

4.2.6 Matching muscle contraction levels for intact and deafferented comparison

Comparison of the force responses to muscle stretch before and after deafferentation can only be performed at matched contraction levels because of the strong dependence of muscle stiffness on the mean background force. As shown in Fig. 4.2*A*, the intrinsic muscle stiffness (deafferented force response amplitude normalized by stretch amplitude) increases nearly linearly with muscle force. For each cat such an intrinsic stiffness vs. force relationship was determined from the averaged responses to stretch for the 8 bins of background force. Then, to estimate the intrinsic muscle stiffness at a force level obtained in the intact decerebrate or spinal cat, an interpolation was done using a linear regression through the 8 points of this intrinsic stiffness/background force relationship. The phase advance of the deafferented force response relative to the imposed stretch was not strongly dependent on the mean contraction force (Fig. 4.2*A*, bottom panel), but it was also interpolated as for the stiffness magnitude. Such stiffness/force relations were similar for each stretch frequencies tested, and are summarized in Figs. 4.2*B* and *C*.

4.2.7 Gain calculation

To measure the reflex gain, a two-part experiment was performed, as in the human elbow joint experiments of Bennett *et al.* 1994. Fig. 4.3 demonstrates graphically the calculations performed on the data obtained during locomotion for 2 different stretch frequencies. The data shown are the best fitting sine waves from many stretch cycles, rather than the raw data, allowing phase and amplitude to be unambiguously determined. First, the reflex force response (F_r) to a sinusoidal stretch (L) of the muscle receptors was estimated, by subtracting the force responses before (F_t) and after deafferentation (F_i, intrinsic force; dashed

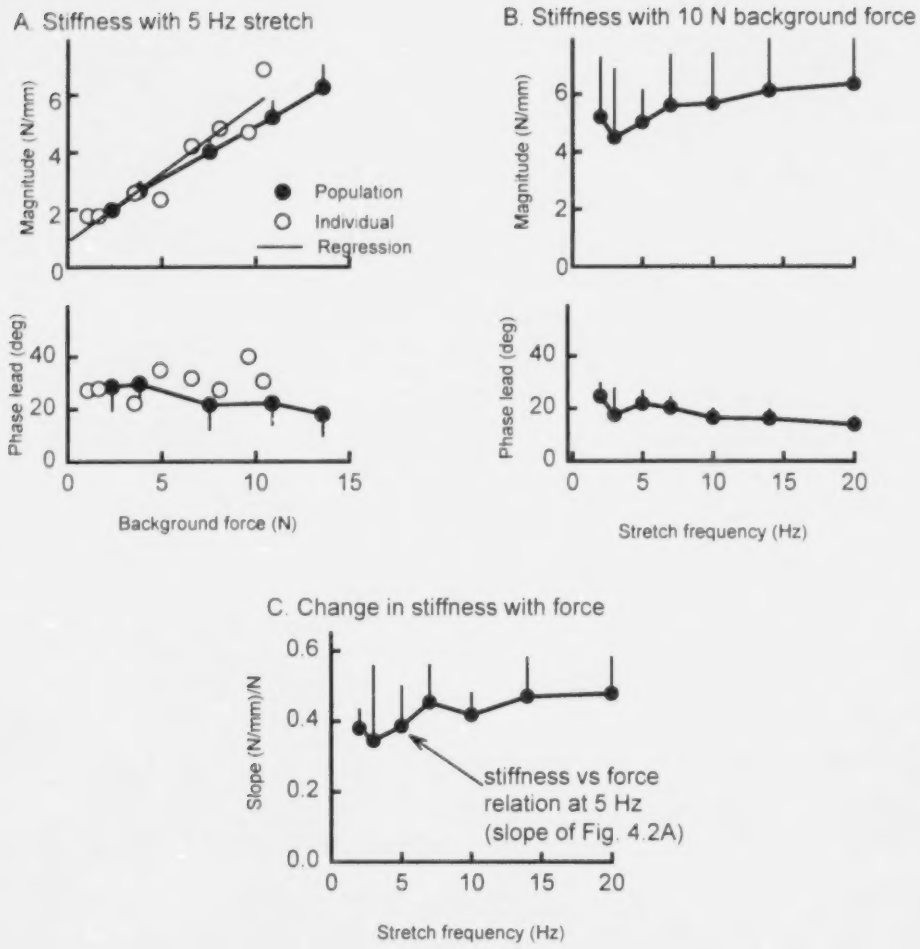

Figure 4.2 - Muscle stiffness after deafferentation.
A. Relationship between muscle stiffness and background force after deafferentation. Upper panel: magnitude of the force response normalized by the stretch amplitude (0.5 mm); lower panel: phase lag of the stiffness relative to the imposed stretch. Stretch frequency: 5 Hz. Open circles: mean stiffness measured from the response to stretch in one cat at 8 different background contraction levels. Thin line: best fitted relation used for interpolation of the stiffness to match the contraction levels measured during locomotion. Filled circles: mean stiffness from all cats (population average). The force range was divided into 5 equal sub-ranges and force responses from all cats that fell in a given sub-range were averaged together. Note the increase in stiffness with force. *B*. Summary of stiffness at different stretch frequencies. Upper panel: mean stiffness at 10 N of background force from all cats. Lower panel: phase lag of the stiffness relative to the imposed stretch. *C*. Change in stiffness with background force and stretch frequency. Slopes of the best fit linear stiffness/background force relations in part *A*, indicating rate of increase in stiffness with force. Mean slopes for all cats. Error bars indicate standard deviation (SD; n=7 cats).

lines in Fig. 4.3). The intrinsic force (F_i) was determined at a matched force level to the intact case, using the interpolation described above. Second, the open loop effect of this reflex force was estimated by determining the change in muscle length (L_o) that would result from applying a copy of the reflex force (F_r) to the deafferented muscle while contracting at the same mean level but allowed to move freely (isotonic). As discussed in the INTRODUCTION, the gain is, by definition, the ratio of the resulting change in length (L_0) over the imposed stretch length (L). For this second part, it is impractical to allow the muscle to contract isotonically during the rapidly varying locomotor contractions, as it would undergo complex length changes, adding the difficulty of stiffness changes set by the length-tension relationship. Instead, we computed the open loop length change (L_o) from our knowledge of the deafferented muscle force responses (F_i) to controlled stretches (L). Using the equation $L_o=F_r/K_i$, we can estimate the change in length due to the reflex force F_r, assuming that the intrinsic stiffness (K_i) remains constant in both intact and deafferented conditions. Since this intrinsic stiffness can be obtained from $K_i=F_i/L$, we get $L_0=F_r/F_i/L$ which, once rearranged, gives us that gain$=L_o/L=F_r/F_i$. The magnitude of the gain is thus equivalent to the ratio of the reflex and intrinsic force response amplitudes, as shown in Fig. 4.3. The phase of the gain is simply the phase shift between the reflex and intrinsic force responses, also shown in Fig. 4.3. Note that due to delay in the reflex pathway the phase lag of the reflex gain is greater for the faster 14 Hz stretch than the slower 5 Hz stretch.

This gain calculation ignores nonlinear effects such as the decrease in muscle stiffness with increased stretch amplitude. For example, if the gain is actually less than unity, the open loop length estimate ($L_o=F_r/K_i$) should be smaller (the estimated stiffness K_i in this case is less than it should be for a smaller stretch). Therefore the computed gain (L_o/L) would be

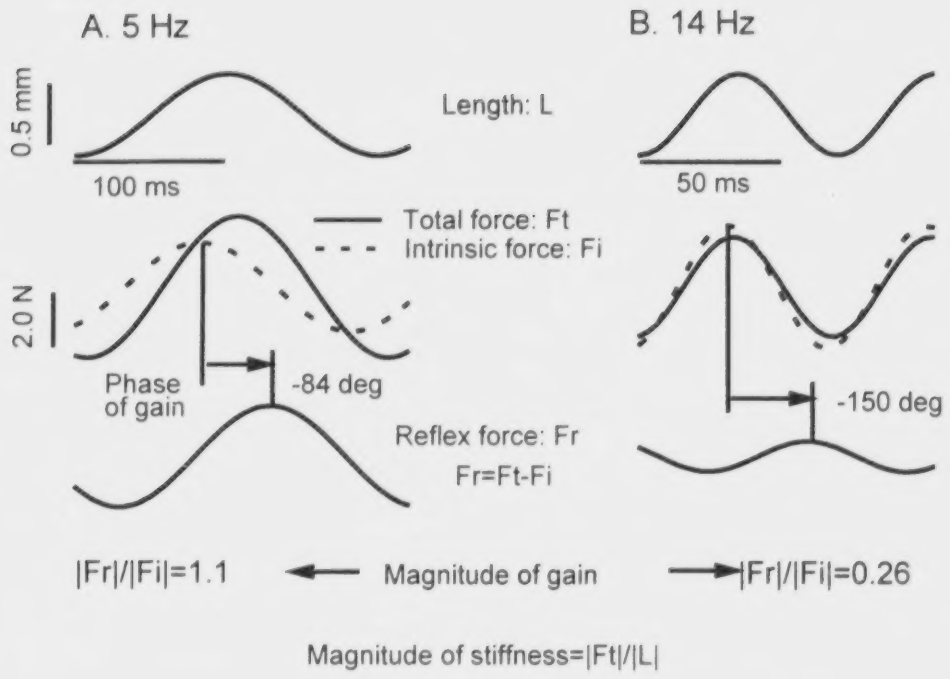

Figure 4.3 - Example of gain calculation.
A. Example of a reflex response to a 5 Hz imposed stretch during the extensor phase of locomotion. The best fit sinusoids are used for calculations, and are shown rather that the raw data (see METHODS). From top to bottom: TS length L, TS force (solid: total intact force F_t, and dotted: deafferented force F_i), and reflex force F_r, the latter computed by subtracting the deafferented from the intact force response ($F_r=F_t-F_i$, Fr and Ft shown on same scale). The gain is $F_r/F_i=(F_t-F_i)/F_i$. Intrinsic force responses used in gain calculations were determined at matched contraction levels by interpolation as described in Fig. 4.2*A*. Note the large, but slightly delayed reflex force (84^0 phase lag), which is comparable in amplitude to the intrinsic muscle force. *B*. Same as in *A* but for the faster 14 Hz stretch. Note the reflex force was delayed by 150^0 from the intrinsic force, which accounted for a reduced total force response.

overestimated. Further, the above derivation ignores the increase in muscle stiffness due to the reflex contraction. However, this problem is minimized by using the deafferented force response (F_i) taken at a force matched to the mean force evaluated during the reflex response. Also, this gain calculation requires the assumption that the force feedback in the intact muscles is negligible.

In summary, during sinusoidal stretching the gain can be estimated simply from the amplitude and phase of the reflex force, relative to the deafferented muscle force modulation (ratio F_r/F_i). Of course, it should be kept in mind that this ratio is intended to be an estimate of the 'open loop gain' which corresponds to the reflex length change produced following a stretch of isolated muscle receptors in a hypothetical 'opened' feedback pathway. This gain estimate only applies in the case where the muscle contracts freely against a constant force (isotonic). Additional external loads that restrict the movement of the muscle (e.g. a spring or an antagonist muscle) will likely decrease the gain.

4.3 RESULTS

4.3.1 Reduction of reflex gain at the onset of locomotion

When the perineal region was stimulated in a Clonidine-treated spinal cat, locomotor-like activity commenced (Pearson *et al.* 1992). Fig. 4.1*A* shows such an example, where the ipsi- and contralateral TS EMG alternate. Stretches imposed on the ipsilateral TS muscles resulted in force and EMG modulations superimposed on the spontaneous background activity (5 Hz stretches shown). In this example, the stretch was turned off twice to show its affect on

background activity. As seen in Fig. 4.1*A*, the background EMG activity prior to locomotion was clearly modulated by the stretch. However, during locomotion, the stretch produced little modulation or change in the gross EMG activity, suggesting that the stretch reflex was much less effective.

Usually even light touch of the perineal region inhibited the TS stretch reflex and this inhibition, at times, occurred without overt locomotor contractions. This inhibition was not simply due to perineal stimulation, as similar stimulation given in the decerebrate cats (not shown) had no effect (assuming perineal cutaneous pathways which inhibit reflexes are not 'opened' during locomotion). Instead, such stimulation can be thought of as switching on the spinal locomotor circuitry, with an associated stretch reflex reduction.

At the end of the extensor activity during stepping there was in some cats a prominent burst of EMG activity (Fig. 4.1*A*). However, this burst was not related to the imposed stretch since it occurred without stretching in both ipsi- and contralateral muscles. Instead, it appeared to be related to the rapid force drop at the end of the contraction, likely causing internal relengthening of the Ia muscle spindles, as discussed in CHAPTER 3. Since this EMG burst was not related to the imposed stretch we ignored it, except for remarking that its presence indicates that the reflexes were not completely inhibited during locomotion.

The force of the muscle was also affected by the locomotor-related reflex inhibition. However, this can only be seen in Fig. 4.1*A* at force levels during walking matched to the low force in the resting state. At higher force levels the responses increased simply as a result of the greater intrinsic muscle stiffness (see METHODS; Fig. 4.2). By selecting and averaging step cycles when the force level is similar to that prior to locomotion (3±1 N), we observed that

both the force and EMG modulations in response to stretch were markedly larger in the resting state (Fig. 4.4*B*) than during locomotion (Fig. 4.4*A*).

The reflex contribution to the force can be seen by comparing the force responses to stretch after deafferentation (intrinsic force; dotted lines in Fig. 4.4, averages made at same mean force level as in the intact state). The difference between the intrinsic and intact force responses gives what we refer to as the *reflex force*. As shown in Fig. 4.4, there was little reflex force during locomotion, compared to the intrinsic force change produced by the stretch. For this specific example, the ratio of the reflex and intrinsic forces (i.e. *reflex gain* magnitude; see METHODS and Fig. 4.3) was 0.3 during locomotion, compared to 1.8 in the resting state. From the 5 cats tested the averaged gain was 0.2±0.1 during locomotion and 0.8±0.3 in the resting state (mean±SD; significant difference at $p<0.05$). The large reflex responses in the resting spinal state are similar to those found in the decerebrate cats (Fig. 4.4*C*; prior to spinalization), at the same low force level (mean gain: 1.1±0.3, n=5).

4.3.2 Reflex gain throughout the step cycle

While the force and EMG responses to stretch were generally small during locomotion, they systematically increased with the mean contraction level. This is seen in the averages at different phases of the step cycle from a cat that had relatively strong stretch reflex responses (Fig 4.5). Averages are shown for each of the 8 phases of the step cycle. Mean force and EMG are plotted vertically to the right of each group of averages to give an indication of the contraction levels during the step cycle. The reflex EMG was larger at high contraction levels (first 4 traces in EMG panel of Fig. 4.5). The reflex force also increased with the contraction,

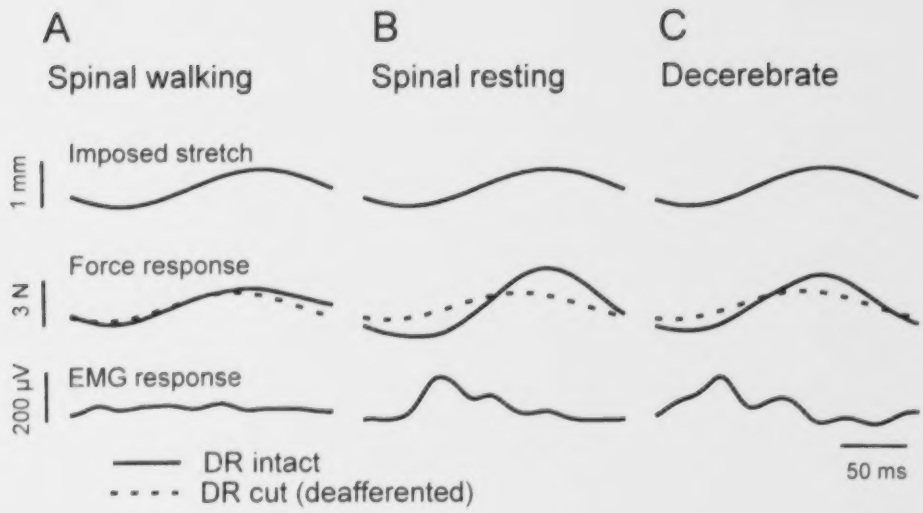

Figure 4.4 - Reduction in stretch reflex at the onset of locomotion.
From top to bottom the solid traces are averaged muscle length (one cycle of stretch), force and EMG. Dotted lines: deafferented responses to the same stretch. Averages under all conditions are from data during low level contractions matched to the resting state. DR: Dorsal roots. *A*. Spinal locomotion; *B*. Prior to locomotion (resting state); and *C*. Decerebrate (resting state). Note the relatively small force and EMG responses to stretch during locomotion, which only marginally increase the intrinsic muscle stiffness (dotted line). All data from a single cat.

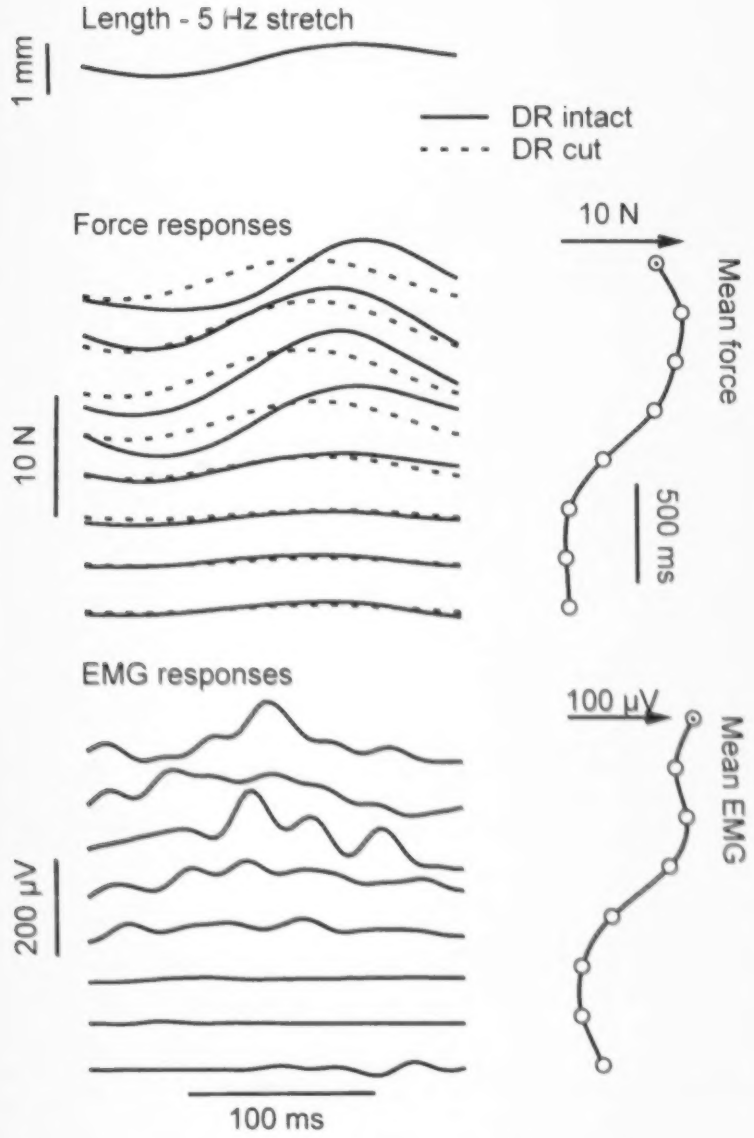

Figure 4.5 - Ensemble of reflex responses throughout the step cycle.
Low stretch frequency condition (5 Hz). Panels from top to bottom are mean TS length (one cycle of stretch), force and EMG for the 8 phases of the step cycle. All traces are shown with the mean level subtracted and are shifted vertically for clarity. The means that were subtracted are shown for each phase of the step cycle by open circles plotted at 90^0 to the right of the force and EMG responses. Force responses to stretch of the muscle after cutting the dorsal roots (DR), at the same mean force levels, are shown with dotted lines. Note that the reflexes produced greater EMG and force responses at higher contraction levels. Simultaneously, the intrinsic muscle force response increased with stronger contractions. Time scale same for all plots.

as seen by the difference of the intact force and the deafferented force (dotted lines in force panel of Fig. 4.5).

The contribution of the reflex to the total force response was assessed by computing the gain ratio, as described above. For the data shown in Fig. 4.5 the reflexes were relatively strong during the extensor contractions, slightly more than doubling the intrinsic muscle stiffness, with a corresponding gain of 1.1 (and a phase lag of 84^0). However, the gain was markedly less than the gain in the resting state (gain=1.8, shown in Fig. 4.4 for the same cat). This lower reflex gain during locomotion was a consistent finding for all cats. Furthermore, as we show next, when all cats are considered there was no step cycle dependence to the reflex gain (it was low and relatively constant).

The reflex gain for each phase of the step cycle is shown in Fig. 4.6, computed from the averages shown in Fig. 4.5. Fig. 4.6*A* shows the averaged force profile during the step cycle and Fig. 4.6*B* the corresponding intrinsic and total stiffness profiles. The hatched region indicates the reflex contribution to the total stiffness. Note the increased reflex contribution at high force levels, contrary to the situation found during tonic contractions (see a similar plot in Hoffer & Andreassen, 1981). The reflex gain (Fig. 4.6C) was, in contrast, only weakly related to the step cycle (and force). In the cat of Fig 4.6 the gain was higher during extension than flexion. However, when gains from all cats are compared the average gain in extension was not significantly higher than the gain during flexion (0.35 compared to 0.22; see Fig. 4.7).

4.3.3 Dependency of reflex gain on contraction level

A corollary of the weak modulation of gain with the step cycle is that there was no clear relationship between gain and contraction level in walking (Fig. 4.7*A*). On average, the

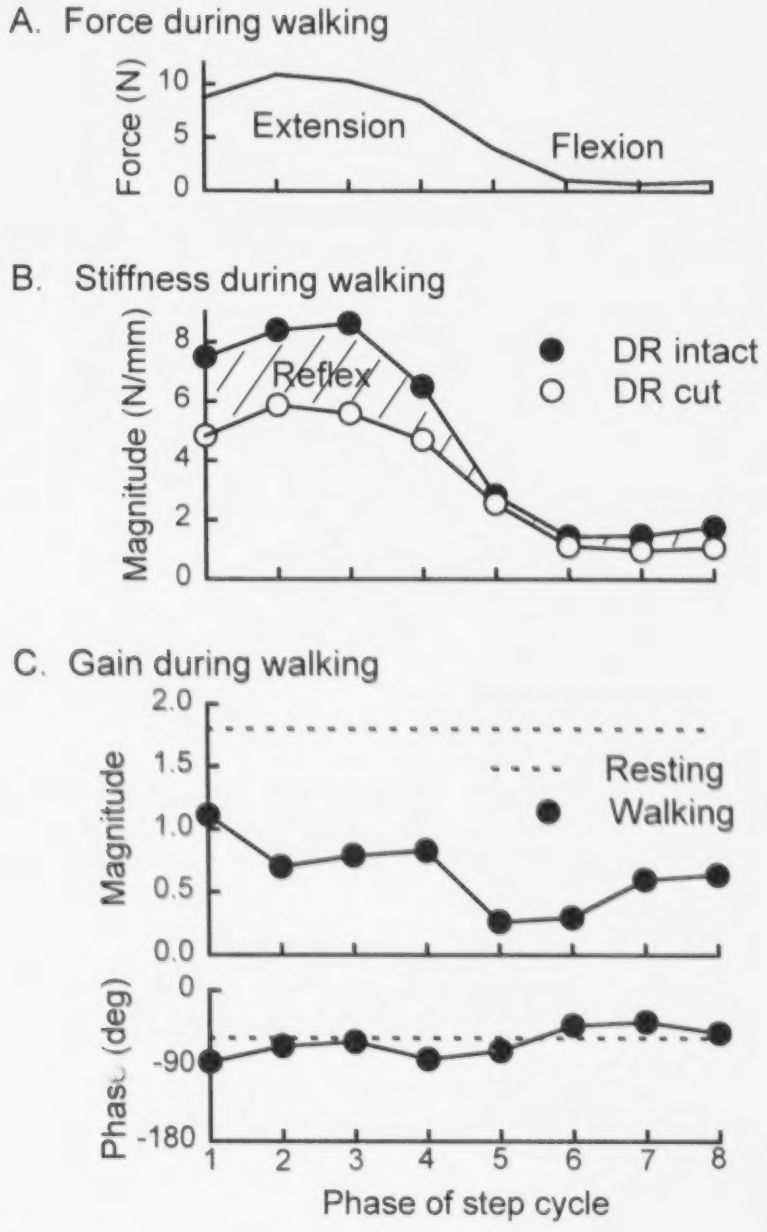

Figure 4.6 - Reflex gain during walking in a spinal cat.
A. Mean forces during 8 phases of the step cycle. Step cycles were normalized with a mean step cycle duration of 1.9 s. *B*. Mean stiffness magnitudes during the step cycle, before (filled circles) and after (open circles) cutting the dorsal roots (DR). *C*. Reflex gains computed from part *B*, using the method described in Fig. 4.3. For reference, the gain during the resting state prior to locomotion is shown with a dotted line (3 N mean force in resting state). Note that the reflex gain (magnitude) during locomotion is markedly lower than in the resting state.

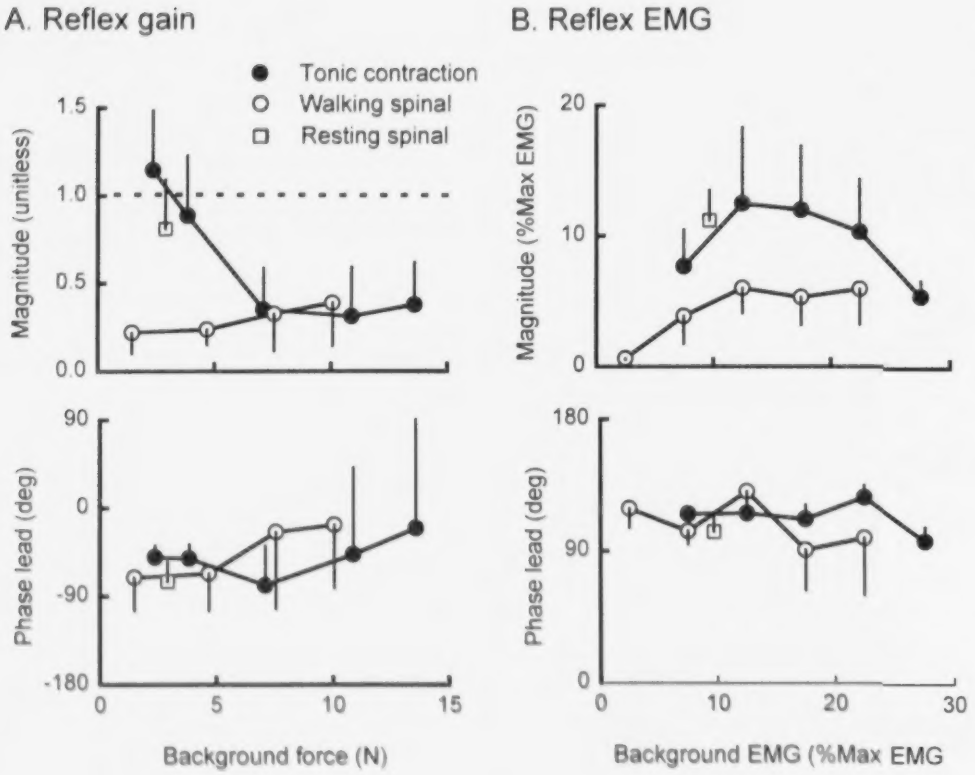

Figure 4.7 - Reflex gain and EMG for 5 Hz stretch.
A. Averaged gain from all cats before and during locomotion (spinal, open symbols) and during tonic contractions (decerebrate, filled circles), plotted as a function of mean background force. Data taken from plots such as shown in Fig. 4.6, and sorted into 5 equally spaced force ranges. The means and standard deviations of the gain are shown plotted at the mean force in each range (thus the uneven horizontal spacing). Error bars: SD. Note the decrease in gain during tonic contractions, and the relatively constant gain during locomotion. Also, note the high gain just prior to locomotion (resting spinal, open square). *B*. Reflex EMG responses computed as the amplitude and phase of sinusoids fitted to the averaged response to stretch, such as shown in Fig. 4.5. Note that the EMG responses dropped with increasing tonic contractions, except for the lowest background contraction level. The opposite was true during locomotion.

gain was low (0.3±0.2, mean±SD), and did not vary significantly with the contraction level ($p>0.1$). This indicates that the reflexes contributed to a small, but significant and approximately fixed fraction (23%) of the stretch-related force during walking ($G/(1+G)$, where the gain $G=0.3$).

The low and constant gain found during locomotor contractions was compared to the gains found during tonic contractions. Tonic contractions at levels comparable to those in locomotion could not be elicited in the spinal state. Thus, we produced such contractions prior to spinalization in the decerebrate state by crossed extensor activation (contralateral CP stimulation; see METHODS). The results obtained show that the reflex gain decreased with the tonic contraction level (Fig. 4.7*A*). As already shown in Fig. 4.4, the reflex gains were large in the decerebrate state during weak tonic contractions (3 N) and comparable to those in the resting spinal state (open square in Fig. 4.7*A*). During increased tonic contractions the gain dropped sharply. At force levels less than 6 N the gain was significantly greater than in locomotion. However, as the contraction level increased above 6 N (maximum of 15 N) the reflex gain decreased to less than 0.5 and was not significantly different than during locomotion ($p>0.1$). In both tonic contractions and locomotion the intrinsic stiffness dominates the gain at high force levels, so the differences in EMG are not seen in the gains (see DISCUSSION).

The EMG during the tonic contraction in Fig. 4.1*B* illustrates one of the main findings in the decerebrate cats: namely, the reflex responses (EMG modulation to stretch) did not increase with the contraction level, in contrast to the situation during locomotion. Rather, responses were large at low contraction levels (left of Fig. 4.1*B*) and decreased at higher contraction levels (note EMG modulation for last 4 stretch cycles at the right of Fig. 4.1*B*, see also Akazawa *et al.* 1982). As discussed in the next section, this situation holds for stretches of

frequencies between 3 and 10 Hz. Fig. 4.1*B* shows data for 10 Hz stretches, since the EMG responses (modulation) were easily seen without averaging. Fig. 4.7*B* (filled circles) shows this decrease in reflex EMG with the contraction for all cats, with a 5 Hz stretch. In contrast, the reflex EMG increased with contraction level during locomotion (Fig. 4.7*B*, open circles).

4.3.4 Dependency of reflex gain on stretch frequency

In walking, when the stretch frequency was increased to 14 Hz (Fig. 4.8), the reflexes no longer resisted the imposed movement (consistent with the behaviour seen during postural contractions; Rack, 1981). Instead the reflexes decreased the total resistance to stretch, as can be seen in Fig. 4.8 by comparing the total (solid lines) to the intrinsic force responses (dotted lines). While the EMG responses to the 14 Hz stretch did not lag the length signal, the slow contraction properties of muscle (excitation-contraction coupling and force development; e.g. Rack, 1981) caused the reflex force to lag the stretch by about half a stretch cycle period (36 ms or 180^0). This is shown in Fig. 4.3*B* where the reflex force is computed for the data in Fig. 4.8 during the extension phase of locomotion. In this case the phase lag of the reflex loop was 150^0 (or -150^0 phase lead).

The averaged phase shifts produced by the reflex loop are shown in the lower panel of Fig. 4.9*A* for all frequencies tested, both during locomotion (open circles) and tonic contractions (filled circles). Fig. 4.9*B* shows the phase of the EMG responses to stretch. Note the clear reduction in phase with increasing frequency, both for the gain and EMG responses. The 180^0 point is marked with a dotted line, as this is the critical point where tremor can potentially occur if the gain is >1 (due to the reflex delay described above for Fig. 4.3*B*; see also Nichols, Stein & Bawa, 1978). Though tremor did occur with appropriate muscle loading

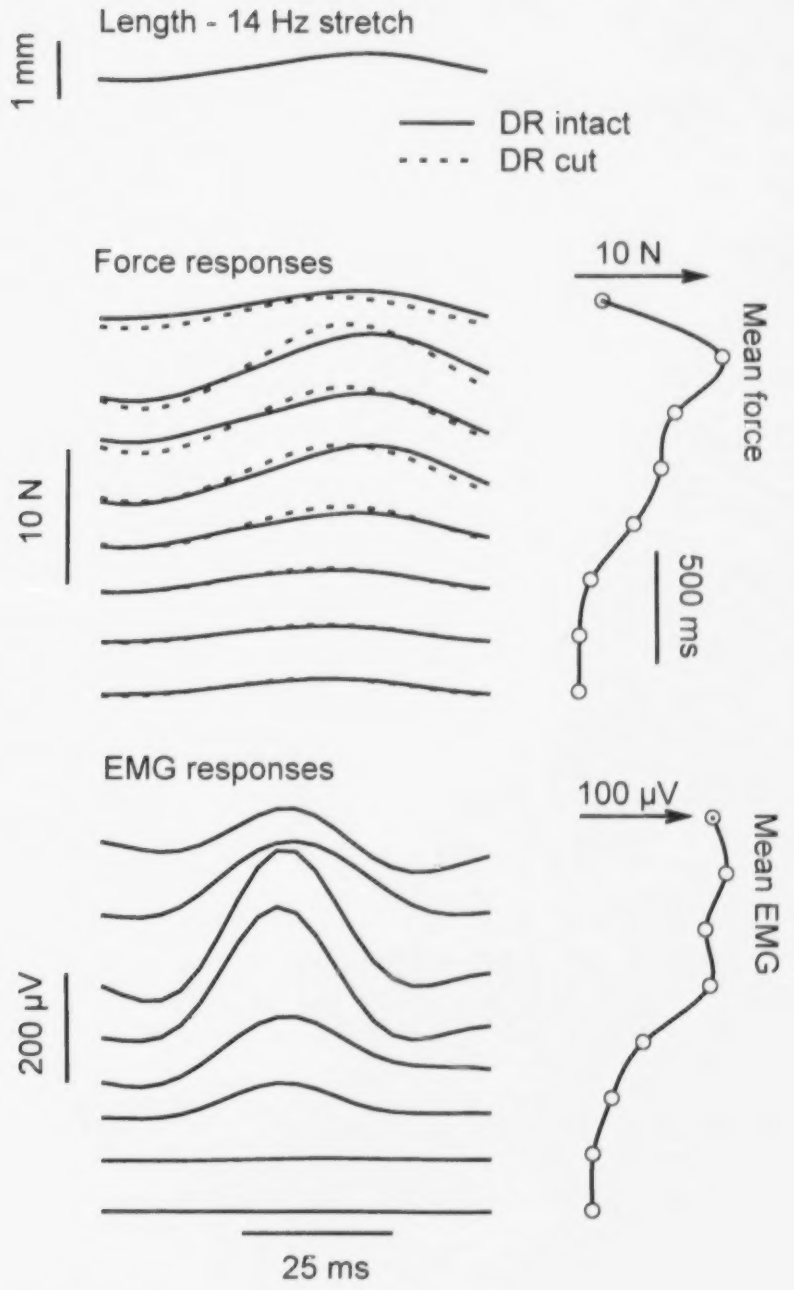

Figure 4.8 - Ensemble of reflex responses throughout the step cycle for a 14 Hz stretch. Same format as in Fig. 4.5. Note that with the reflexes intact, the total force responses (solid lines) are smaller than without reflexes (dotted lines), as explained in Fig. 4.3.

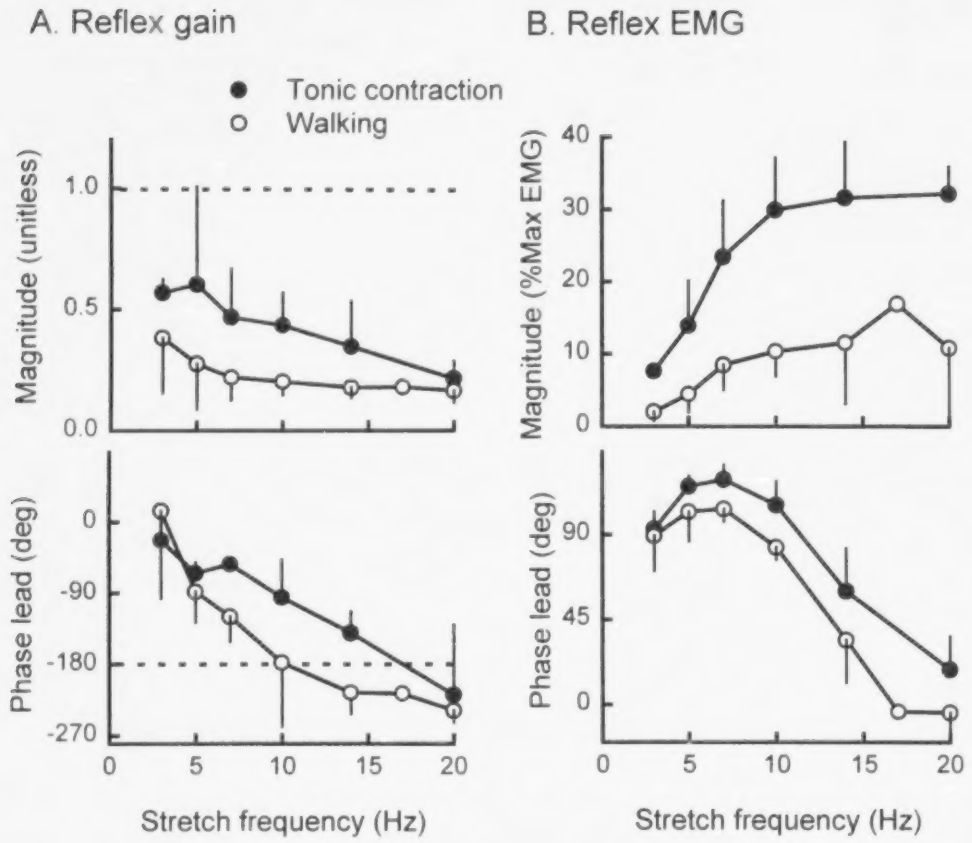

Figure 4.9 - Dependency of reflex gain on stretch frequency.
A. Mean gain magnitude and phase of the stretch reflex at various frequencies, during locomotion (open circles) and tonic contractions (filled circles). Each point represents the mean of data from a 0-15 N range of contraction levels (mean 6.5 N; n= 5 cats). Error bars: SD. Note that the mean gain is always larger during tonic contractions. Also note that the reflex loop phase lag increased with the frequency. The phase lag is greater during locomotion, and crosses the critical 180^0 line (dotted line) just above 10 Hz. *B*. EMG responses (modulation and phase) at various stretch frequencies. Same format as in part *A*, but means of EMG responses from 5 cats at a 10% contraction level (%Max EMG). Similarly, the reflex response magnitudes and phases are greater during tonic contractions.

in the decerebrate cat, the gain was never as great as 1 at this critical point, likely because the gain was measured with a larger stretch amplitude than the tremor amplitude (see DISCUSSION). Tremor could not be induced during locomotion because the reflex gain was too low.

An unexpected finding, demonstrated in the phase plot of Fig. 4.9*A*, was that during tonic contractions there was a greater phase lead (smaller phase lag) than during locomotion (the difference for 7-14 Hz was significant at $p<0.05$). This slower response in the reflex during locomotion suggests that additional slower reflex pathways may be involved (see DISCUSSION). Changes in muscle properties could not account for this finding, since this phase difference was also seen in the EMG responses (Fig. 4.9*B;* unless there were differences in recruitment of soleus and gastrocnemius muscle in tonic contractions and locomotion).

At all frequencies the magnitude of the reflex gain during locomotion was less than the gain during tonic contractions (significant difference for 3-14 Hz, $p<0.05$), as already discussed for the 5 Hz stretches. The same conclusion holds for the EMG responses. However, the averaged gain was reduced at higher frequencies, both during locomotion and tonic contractions. An explanation of this result is required since the EMG responses to stretch increased significantly with the stretch frequency. At high frequencies the more frequent reflex muscle activation produced a more fused contraction, and thus produced less modulation in force (due to the lowpass filter properties of the muscle; Bawa, Mannard & Stein, 1976). Therefore at high frequencies the reflexes contributed to a steady increase in the force ('vibration' reflex), which is not reflected in the gain calculation.

Fig. 4.9 shows only the mean gains during a wide range of contraction levels, which is all that is required for locomotion, as the gain is constant throughout the step cycle (see above

and Fig. 4.10). Fig. 4.9 does not however reveal the change in gain during increasing tonic contractions, such as shown in Fig. 4.7*A*. Our results showed that at the lowest tonic contraction levels the gain often exceeded 1 for stretch frequencies of 3-5 Hz. To further quantify the relationship between gain, background force and stretch frequency we fitted a linear regression line to the gain/background-force relation for each cat (giving a 'slope' and 'offset', e.g. Fig. 4.7*A*, top panel). These regression 'slopes' are shown as a function of the stretch frequency in Fig. 4.10. Together with Fig. 4.2, Figs. 4.9 and 4.10 provide a complete description of our results, and can be used to reconstruct (simulate) the muscle behaviour.

During tonic contractions (Fig. 4.10*A*) the gain was negatively related to force for 3-10 Hz stretches (negative slopes, significant at $p<0.05$). Fig. 4.10*B* shows a similar analysis for the EMG responses, where the slopes of the reflex/background EMG relations (such as in Fig. 4.7*B*, top panel) are significantly negative at 5-7 Hz. This is consistent with the gain results, though over a narrower frequency range. During locomotion, the slopes of the gain/background force relations were remarkably low, not significantly different from zero, at all stretch frequencies ($p>0.1$), again indicating that the reflex gain was relatively constant.

4.4 DISCUSSION

4.4.1 Locomotion and automatic gain control

We have shown that at the onset of locomotion in the Clonidine-treated spinal cat there is a marked reduction in the reflex gain. Furthermore, variations in contraction level during locomotion are not associated with significant changes in the reflex gain. The relatively

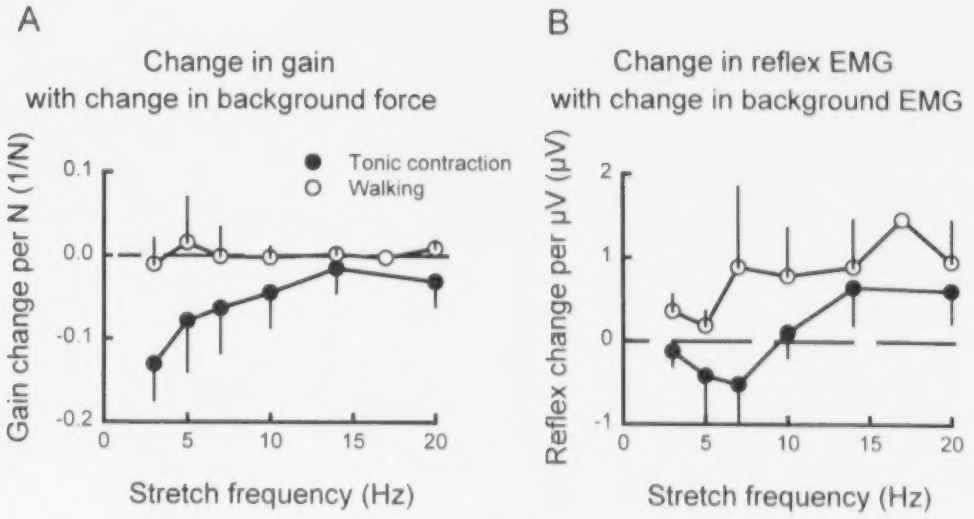

Figure 4.10 - Change of reflex gain and EMG with contraction level and stretch frequency.

A. Slopes of gain/background force relationships, as shown in Fig. 4.7*A*, for various stretch frequencies (mean slope±SD, n=5 cats). For each frequency the slope was computed by a linear regression on the gain/background force relation, and indicates the increase in gain that occurs with a 1 N increase in the background contraction level. Note that during tonic contractions (filled circles) the gain decreased with increasing force (negative slopes). During locomotion (open circles) the gain did not change with the force level at all frequencies (zero slope). *B*. Slopes of reflex EMG/background EMG relationships, as shown in Fig. 4.7*B*, for various stretch frequencies. Same format as in part *A*, though slope indicates change in reflex EMG amplitude with a unit increase in background contraction level. Note the negative slopes during tonic contractions at low stretch frequencies, and the positive slope during locomotion, only the latter being consistent with the 'automatic gain control' concept.

constant reflex gain is surprising, considering the clear positive relationship between the reflex EMG and the contraction level (due to 'automatic gain control', Matthews, 1986; see also Akazawa *et al.* 1982). This result can be explained by noticing that the intrinsic muscle stiffness also increases with the contraction. Since the reflex stiffness increases proportionally to the intrinsic stiffness, the ratio of these two stiffnesses (i.e. the gain) remains constant. Functionally, this implies that at higher contraction levels during locomotion, reflex contractions are stronger, but do not produce a greater effect on the final muscle movement because the muscle itself has greater resistance to movement.

The control of the reflex gain is considerably different during tonic contractions. The reflex gain is large at low contraction levels (about 1 at 5 Hz) and decreases at higher contraction levels. This is consistent with the previous findings that reflex EMG and force responses to stretch are negatively related to the contraction level (Akazawa *et al.* 1982), or at least do not increase positively with the contraction (Hoffer & Andreassen, 1981; soleus), as would be required to maintain a constant gain. These cat data are also consistent with human reflex gain (Bennett *et al.* 1994) and EMG measurements (e.g. in standing, Capaday & Stein, 1986). The net effect of this gain control is that the total stiffness remains approximately constant for moderate level contractions (e.g. Hoffer & Andreassen, 1981). However, this situation is not the only mode of control during tonic contractions (Stein & Kearney, 1995). In the decerebrate cat, brain stem activation can significantly reduce reflex action (Hoffer & Andreassen, 1981; Nichols & Steeves, 1986). On the other hand, jaw reflex gains in primates are several times greater than those in cat hindlimb (Goodwin *et al.* 1978), which is remarkable, considering the likelihood of reflex induced tremor even with the rather low gains found in the cat hindlimb.

4.4.2 Central mechanisms in gain reduction

The lower reflex gain during locomotion may depend on a number of central changes, in addition to the effects of intrinsic muscle properties. For example, a predominantly static fusimotor drive is initiated at the onset of locomotion, which reduces primary afferent sensitivity and thus reflex responses, as discussed in CHAPTER 3. Also, primary afferent terminals are likely presynaptically inhibited, particularly during the flexion phase of locomotion, as terminal depolarization has been reported during locomotion (or locomotor-like FRA contractions) in DOPA-treated spinal and decerebrate cats (Andén *et al.* 1966, Gossard *et al.* 1991). It should be remembered though that only the extensors were stretched in the current experiments, and flexor, rather than extensor, Ia afferents produce presynaptic inhibition peripherally (Eccles, Magni & Willis, 1962; Eccles, Schmidt & Willis, 1962). Postsynaptically, with all other factors being equal, the reflex responses should become greater at higher contraction levels ('automatic gain control', see above and Akazawa *et al.* 1982). Further, changes in motoneurone membrane properties that occur from monoaminergic activation (e.g. DOPA and Clonidine) probably further alter the reflex responses (e.g. Conway, Hultborn, Kiehn & Mintz, 1988), but the details remain unclear.

Finally, polysynaptic group Ib and II reflex pathways appear to become operable during locomotion, and should in principle augment the reflex responses measured (e.g. Gossard & Hultborn, 1991; Perreault *et al.* 1995). Moreover, during locomotion, Ib afferents can be as sensitive as the Ia afferents to the test stretches used in the present study (see CHAPTER 3), indicating that the Ib input is at least available to support such polysynaptic reflexes. Consequently, the increased phase lag seen in the stretch reflex in the locomoting cat (Fig. 4.9) may result from responses mediated by Ib afferents, which have slower response dynamics than

the Ia afferents (with a 4 Hz stretch, Ib afferent firing lags the Ia afferent firing by about 50^0, see CHAPTER 3, see also Chen & Poppele, 1978, and Houk & Simon, 1967). Of course, differences in recruitment of soleus and gastrocnemius muscles may also affect reflex lags (see RESULTS).

During tonic contractions the reflex reduction is likely related to a strong static fusimotor coactivation (see CHAPTER 3). Interestingly, the reflex reduction only occurred in the 3-10 Hz frequency range (Fig. 4.10), consistent with the finding that static fusimotor activation was more effective in this frequency range (Taylor *et al.* 1985). In contrast, our results showed a pronounced reflex reduction at all frequencies during locomotion, suggesting the presence of additional inhibitory mechanisms (e.g. presynaptic).

4.4.3 Comparison to other locomotor preparations

The generality of the present gain estimates to other walking preparations is unclear because no comparable deafferentation studies have been performed during locomotion. It is of interest though, that in chronically spinalized cats treated with Clonidine to facilitate locomotion, corrective movements for obstacles are impaired, as are tendon tap responses (Chau, Provencher, Lebel, Barbeau & Rossignol, 1993). Clonidine, rather than locomotion, is not solely responsible for the reflex reduction since: (1) reflexes in the resting Clonidine treated spinal cat are comparable to those in the decerebrate cat (high gain) and (2) in other spinal locomoting preparations reflexes are likely reduced. For example, the increased Ia afferent presynaptic inhibition and decreased Ia afferent sensitivity in the DOPA-treated spinal cat (Andén *et al.* 1966; see also CHAPTER 3) suggest that this locomotor-like preparation also has low reflex gains. Similarly, the reflex gain is probably reduced in the locomoting decerebrate

cat since both EMG and force responses to stretch are reduced (at comparable contraction levels) with the onset of locomotion (Akazawa *et al.* 1982). Additionally, in humans, the H-reflex is markedly reduced at low contraction levels during locomotion. At high contraction levels, the reflex is increased only to levels comparable to standing (Capaday & Stein, 1986). However, in the latter case, the gain is presumably much smaller than the gain at low contraction levels during standing because of the greater muscle stiffness (as discussed above).

4.4.4 Functional implications

The results of the present study allow us to re-examine the question of how much the reflexes contribute to locomotion. Previously, Severin (1970) blocked the fusimotor axons peripherally, and found that during locomotion the total triceps surae EMG was reduced by 50%, presumably because of reduced muscle spindle feedback. However, this number may be an overestimate of the reflex contribution, since some of the α-motoneurones may have also been partially blocked. Yang *et al.* (1991) stretched the ankle during stance to estimate the velocity sensitivity of the soleus reflex (units of EMG/deg/s). They then multiplied this velocity sensitivity by the ankle velocity during normal undisturbed locomotion to predict the EMG produced by the reflex, which was found to be 30-60% of the total soleus EMG during normal stance. This estimate may also be high because of several nonlinearities in the human ankle reflexes (Stein & Kearney, 1995).

From the averaged gain of 0.3 obtained during locomotion in the present study, we can estimate that the reflexes contribute to 23% of movement-related force (stretch) of the TS muscles during locomotion. In one cat (Fig. 4.6) the gain during the extension phase of locomotion became as high as 1, suggesting that the reflexes can produce 50% of the stretch-

related force during stance. The total force is, however, greater than the stretch-related force since the muscles can, of course, produce force without lengthening (as in our isometric 'walking' situation). Thus, the reflexes produce, on average, less than 23% of the total ankle force. Possibly higher gains may occur in the normal intact animal due to: (1) the lack of Clonidine, and (2) heteronymous reflexes (e.g. Sinkjær & Hoffer, 1990). Furthermore, larger or smaller stretch amplitudes would respectively decrease or increase the gains (see METHODS and Nichols *et al.* 1978).

In summary, the results from our study suggest that prior to locomotion the reflexes can contribute to about half of the total force during low level postural corrections (the reflex loop gain is about 1 in spinal or decerebrate cats, as in humans). With stronger contractions the reflex gain is significantly reduced. The increased intrinsic stiffness occurring with stronger contractions allows the muscle to more easily resist unexpected disturbances and reduces the need for a strong stretch reflex. The lower reflex gain during both postural and locomotor contractions is of benefit from the standpoint of stability. When reflexes are unusually large during locomotion, as in spastic patients, tremor can occur at such critical times as foot contact. Further, during rapid movements, reflexes may be too slow and even act inappropriately (e.g. Grillner, 1972). Finally, during the flexion phase of locomotion a stretch reflex in ankle extensors would resist the desired movement and be counter-productive. Therefore, the reflex modulation seen in locomotion in preparations as diverse as spinal cats and intact humans makes good sense in terms of the functional requirements in various phases of the movement.

Post Scriptum

This chapter has been accepted for publication:

BENNETT, D.J., DE SERRES, S.J. & STEIN, R.B.
Gain of the triceps surae reflex in decerebrate and spinal cats during postural and locomotor activities.
***Journal of Physiology*, (in press)**

During the realization of this project, I was involved in the data acquisition procedure (for all the experiments), I participated actively to many discussions concerning processing of the data, and collaborated in the preparation of the manuscript.

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CHAPTER 5

STRETCH REFLEX IN CAT TRICEPS SURAE MUSCLES DURING TONIC AND PHASIC CONTRACTIONS WITH ISOMETRIC AND COMPLIANT EXTERNAL LOADING

5.1 INTRODUCTION

Despite a large number of studies performed on the stretch reflex, details of its mechanism and control still remain unclear. When considering the predominant monosynaptic component of this reflex, many investigators looked at the change in the amplitude of the H-reflex, the electrical equivalent of a tendon jerk, in a variety of tasks in humans (for review see Schieppati, 1987; Stein, 1995). Modulation of the H-reflex was observed throughout cyclic movements such as locomotion (Capaday & Stein, 1986; Crenna & Frigo, 1987; Yang & Whelan, 1993), or pedalling (Brooke, McIlroy & Collins, 1992). During walking, the H-reflex size was closely related to the amount of EMG activity in the muscle, facilitated during the stance phase and suppressed during the swing phase. A similar modulation of the H-reflex was also observed in decerebrate cats (Akazawa, Aldridge, Steeves & Stein, 1982).

These results first suggested that the amplitude variation of the H response might only reflect the change in the level of excitability of the motoneuronal pool (allowing more motoneurones to be ready to fire in response to a stimulus). But the readiness in the

motoneuronal pool cannot solely account for this modulation. It was shown that for a comparable level of muscle activation, the H-reflex was attenuated in walking compared to standing, and in running compared to walking (Stein & Capaday, 1988; Brooke, Collins, Boucher & McIlroy, 1991; Edamura, Yang & Stein, 1991). Furthermore, Yang and Whelan (1993) showed that, even with subjects trained to walk with an extra burst of activation of the soleus muscle during the swing phase, the H-reflex remains attenuated in that phase.

In studying the H-reflex, two components of a stretch reflex loop are missing: the mechanical properties of the muscle shaping the reflex contraction and the influence of the fusimotor drive on the spindle afferent sensitivity. The stretch reflex was previously examined in decerebrate cats when locomotion was induced by electrical stimulation of the mesencephalic locomotor region (Akazawa *et al.* 1982). Pulse stretches were applied to the soleus muscle at various phases of the step cycle and the results obtained showed a deep modulation in the stretch reflex, matching the variation observed in the H-reflex for the same muscle. The results also showed that the reflex varied in parallel with the intrinsic muscle stiffness, thus being positively related to the background force. Interestingly, a reverse relationship was observed during tonically active muscle (spontaneous activity), the force produced in response to stretch decreased with increasing levels of background activity.

In the present study, pulse stretches (10 ms in duration) were applied to the triceps surae (TS) muscles of decerebrate cats during tonic contractions produced by stimulation of the contralateral common peroneal nerve (crossed extensor reflex). Identical stretches were also applied after spinalization, during locomotion generated by the administration of

Clonidine. Our results reiterated the negative relationship between reflex and background forces observed during tonic contraction and demonstrated that the reflex is modulated during locomotion even in a spinal preparation.

Due to technical difficulties, short stretches have rarely been used in human studies (Stein, Hunter, Lafontaine & Jones, 1995; Stein & Kearney, 1995). Instead, ramp-and-hold perturbations were widely utilized (Allum & Mauritz, 1984; Sinkjær, Toft, Andreassen & Hornemann, 1988; Carter, Crago & Keith, 1990; Toft, Sinkjær & Andreassen, 1989; De Serres & Milner, 1991; Toft, Sinkjær, Andreassen & Larsen, 1991; Doemges & Rack, 1992; Carter, Crago & Gorman, 1993). Likewise, many studies performed on cats, used a ramp-like stretch (Nichols & Houk, 1976; Hoffer & Andreassen, 1981; Nichols, 1985; Sinkjær & Hoffer, 1990; Lin & Rymer, 1993). The use of a ramp-like stretch evoked a longer latency reflex in addition to the short-latency reflex. In the present study, ramp-and-hold perturbations were also applied to stretch the TS muscles of decerebrate cats, permitting a comparison between dynamic and static reflex responses.

Throughout the present experiments, the torque motor used to stretch the muscles behaved like a spring of a preset stiffness (from isometric to very compliant). This type of compliant loading simulates more closely the normal physiological conditions observed in an intact preparation: in response to sudden stretch, muscles generally contract against elastic tissues which may include antagonistic muscles with various degrees of activation. Moreover, during a task such as locomotion, the stiffness of the external environment varies greatly throughout one cycle, from very smooth while the foot is in the swing phase to very hard at the time of ground contact. Such external loading properties have been

used to study the mechanisms of muscle contraction in cats (Bawa, Mannard & Stein, 1976a,b) and the behaviour of short-latency reflexes in the human elbow flexor muscles (Stein *et al.* 1995). In this latter study, a dramatic increase in reflex shortening accompanied by an attenuated reflex force was observed when the load was made more compliant (less stiff). With the use of an external load featuring a range of stiffnesses in the present study, we confirm these effects of load compliance on the reflex responses.

In a series of experiments with the same cat preparations as used in the present study, a sinusoidal stretch was applied to the TS muscles and the gain of the reflex loop was estimated (see CHAPTER 4). They showed that the gain was markedly lowered during walking in the spinalized cats, when compared to a similar level of contraction in tonically activated muscles. Surprisingly, the gain did not vary significantly throughout the step cycle, showing no clear relationship between gain and contraction level. In fact, the reflex force and electromyographic responses to stretch increased with the contraction level, but their effect on the total reflex gain was cancelled by an associated increase in the intrinsic muscle stiffness.

With pulse inputs applied to the human ankle joint, superimposed on larger random perturbations, Stein and Kearney (1995) showed that the reflex responses decreased in a graded manner with increasing amplitude or bandwidth of the random perturbations. These results suggested that the gain of the reflex loop would be enhanced if calculated when short discrete pulses are applied rather than continuous perturbations such as sinusoidal stretches (see CHAPTER 4). Computer simulations were performed on the data obtained in the present study in order to verify this result. These simulations also permitted

a more detailed analysis of the effect of external load stiffness on the stretch reflex loop gain.

5.2 METHODS

5.2.1 General preparation

Experiments were performed to examine the reflex response to stretch of the triceps surae (TS) muscles of the left hindlimb in 7 male cats (3.9 ± 0.6 kg). For all cats, the muscle responses to stretch were recorded for 3 different conditions: (1) after premammillary decerebration, (2) after spinalization between T12 and T13 vertebrae, and (3) after deafferentation by sectioning the ipsilateral L5-S2 dorsal roots. Five of these seven cats were the same animals used to study the gain of the stretch reflex with sinusoidal stretches (see CHAPTER 4).

5.2.2 Nerve and muscle preparation

Under general anaesthesia (Halothane 1.5-2.5% mixed with 95% O_2, 5% CO_2), muscles from both hindlimbs were extensively denervated sparing only the tibial nerve (supplying TS muscles) on both sides, and the common peroneal (CP) nerve (supplying ankle flexors) on the contralateral side. A nerve cuff made of hydrophilic vinyl polysiloxane impression material (Reprosil, Dentsply International Inc., Milford, Delaware, USA) was used to maintain two copper wires around the ipsilateral tibial nerve for its stimulation. A nerve cuff along with wire electrodes was also placed around the CP

nerve of the contralateral hindlimb for stimulation. The calcaneum bone was sectioned in order to free the tendon of the triceps surae muscles from the foot. An inelastic silk thread (#5) was tied either through a hole drilled in the calcaneum bone or around the tendon proximal to the bone. The cat was mounted in a stereotaxic frame, the ipsilateral hindlimb was immobilized by two bone screws pressing against each side of the femur just above the denervated knee and by a foot clamp. The TS tendon thread was attached to a muscle puller. A single pulse stimulus was delivered via the tibial nerve electrodes throughout the experiment to set the initial muscle length at the position of maximal twitch force. This muscle length (about 135^0 ankle position in dorsiflexion) was selected since it was at the peak of the length-tension curve (Rack & Westbury, 1969), where muscle stiffness changes minimally with small stretches.

5.2.3 Tonic and phasic activation of the TS muscles

A crossed extensor reflex was induced by stimulating the common peroneal nerve of the contralateral hindlimb in the decerebrate preparation (stimulation with pulses of 0.2 ms at 50 Hz with amplitude graded to generate different contraction levels, see Fig. 5.1*A*). After spinalization, locomotion was generated upon administration of the opiate antagonist Naloxone (25 mg/kg; Sigma Chemical Co., St.Louis, MO, USA) along with the α_2 noradrenergic agonist Clonidine (50-200 mg/kg; Sigma Chemical Co.). Rhythmicity was initiated by manual stimulation of the skin of the perineum. Step cycles occurred at 0.5-1 Hz for periods of 1-2 minutes (Fig. 5.1*B*), and were usually not entrained by the manual stimulation (Pearson, Ramirez & Jiang, 1992). The stretch was

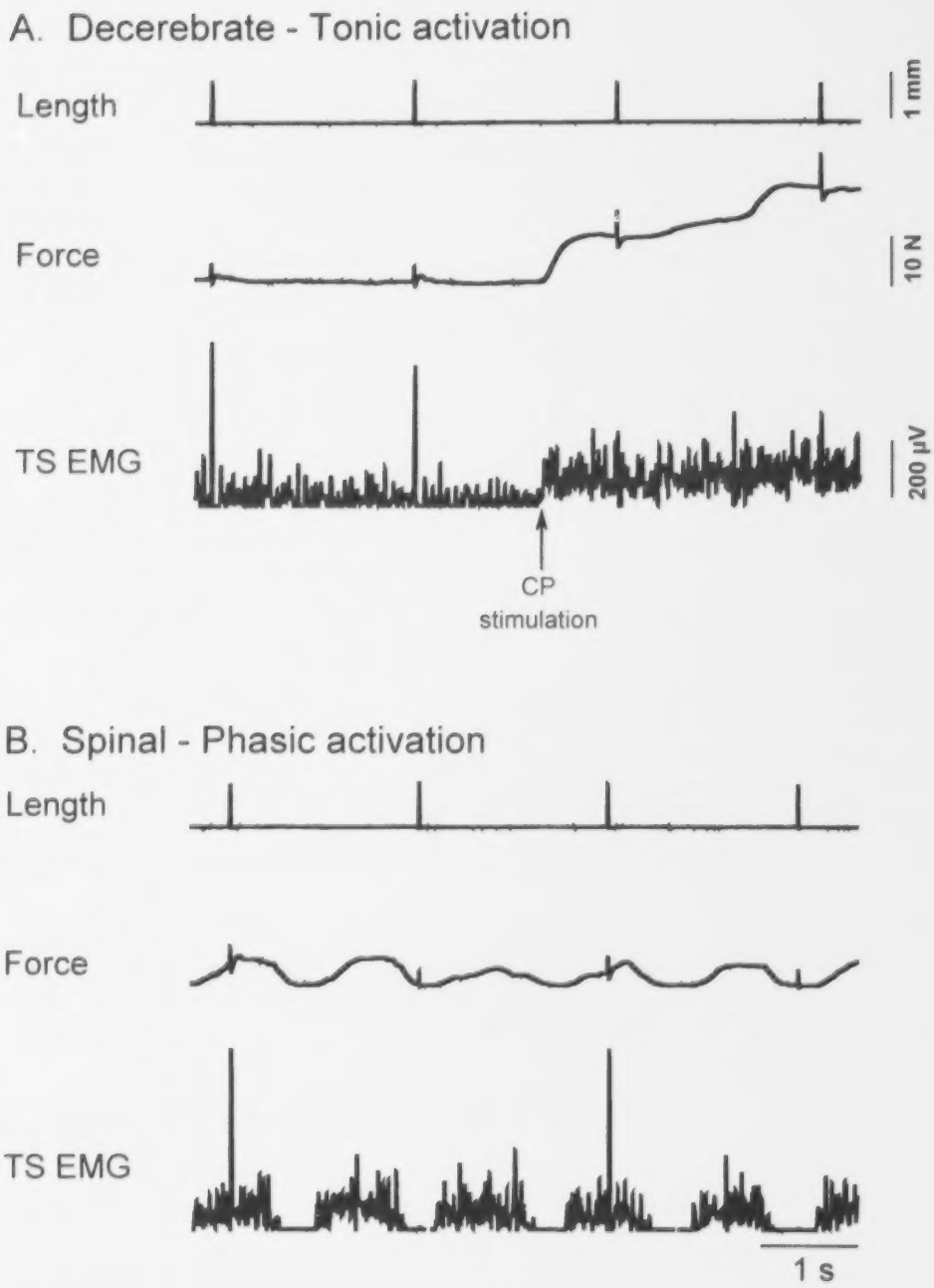

Figure 5.1 - Reflexes during tonic and phasic contractions.
A. Stretch reflex responses to pulse stretches before and during contractions produced by contralateral CP nerve stimulation (vertical arrow marks onset of stimulation) in a decerebrate cat. Only the beginning of the tonic contraction is shown. From top to bottom the traces are respectively: TS length, force and EMG. Data recorded with an isometric load. *B*. Stretch reflex responses to pulse stretches during locomotion in a spinal cat. Same format as in part *A*.

applied at a frequency of 0.7 Hz during the tonic or the rhythmic activation of the TS muscles.

After deafferentation, both types of muscle activity (tonic and phasic) were produced, using the same methods. In some cases, the crossed extensor reflex failed to produce tonic activity in the TS muscles. When that happened, a direct activation of the muscles was generated by stimulation of the tibial nerve (pulses of 0.1 ms at 50 Hz).

5.2.4 Stretch application

Stretches of two different waveforms were applied by a servo-controlled motor (310B, Cambridge Technology Inc., Watertown, MA, USA): a short pulse (for both tonic and phasic background contractions) and a ramp-and-hold stretch (for tonic background contractions). The short pulse lasts for about 10 ms. The ramp-and-hold stretch had a lengthening phase of 30 ms, followed by a 240 ms plateau and a shortening phase of 30 ms. Stretches of 1 and 3 mm in amplitude were applied for both waveforms. The above amplitudes correspond to a change in length on a straight line tangent to the course path described by the tip of the motor manipulandum when it rotates to stretch the muscles. Since the thread used to attach the TS muscle to the tip of the manipulandum formed an angle of approximately 70^0 with this tangent (varying from one cat to another), the actual stretch amplitudes were slightly smaller than the values stated above.

By varying length and velocity feedback (via the motor controller), the motor puller (external load) could behave like a spring of a particular stiffness (from isometric to very compliant). The motor compliance was varied throughout the experiments with values of 0.2, 0.5 and 1.5 mm/N, in addition to the isometric condition. Only the pulse

stretch of 1 mm in amplitude was used with these elastic loads. When loads were compliant, it was necessary to augment the nominal amplitude of the stretch (commanded through the motor controller), in order to keep the actual muscle stretch at about 1 mm.

5.2.5 Signal recording and averaging

Intramuscular electrodes, consisting of two Teflon-coated silver wires, were inserted with hypodermic needles into the TS muscle group of the ipsilateral hindlimb (one electrode in soleus and the other in lateral gastrocnemius), for recording of EMG activity. Both the force and the length signals were measured from transducers built into the motor. Force, length and EMG signals were amplified, filtered with a bandpass of 10-300 Hz, and stored on a video tape for later analysis. These signals were also displayed on oscilloscopes for monitoring throughout the acquisition period. A brief impulse was delivered through the D/A converter just prior to the application of the stretch and this signal was also stored on tape. Digitization of the signals (sampling rate of 300 Hz) was performed by an Axotape A/D converter (Axon Instruments, Inc., Foster City, CA, USA). Prior to digitization, the EMG signal was further amplified, rectified and low-pass filtered at 100 Hz.

Force, length and EMG responses to stretch were averaged for each experimental condition, separating the data recorded during tonic contraction from the data obtained during rhythmic activity. The averaging process was triggered by the brief impulses delivered prior to the application of the stretch. In the case of tonic contractions, the total range of muscle activation was divided in six intervals of equal size for averaging. For each signal, responses occurring at a similar background force level were averaged

together. In the case of phasic contractions, the total duration of the step cycle was divided into eight intervals of equal size. For each signal, responses occurring at the same time in the step cycle were averaged together. Further analysis was performed on the averaged traces for each signal (MATLAB software from MathWorks Inc., Natick, MA, USA) as explained below.

5.2.6 Data analysis

PULSE STRETCH AGAINST ISOMETRIC LOAD. Two upward deflections were seen on the averaged force trace (Fig. 5.2*A*) in response to a pulse stretch of the muscles: a transient deviation corresponding to a direct mechanical effect of stretching the muscle, and a slower deflection representing the force produced by the slower reflex response. The intrinsic muscle stiffness was evaluated by dividing the force change directly related to the stretch (amplitude of transient deviation) by the measured amplitude of the stretch. Reflex force was calculated by subtracting the deafferented force responses from the responses obtained in the intact conditions (decerebrate or spinal). Due to the strong dependence of intrinsic muscle stiffness on the mean background force (e.g. Hoffer & Andreassen, 1981), it was important that this subtraction occurred for matched levels of background force. Prior to subtraction, the averaged data from the deafferented condition were therefore adjusted so that the background force level and the intrinsic muscle stiffness matched those measured in the decerebrate or spinal condition (Fig. 5.2*A*). The maximal value of the subtracted force trace was computed as the reflex force response. A distinct short-latency EMG volley appeared in response to the pulse stretch. The size of

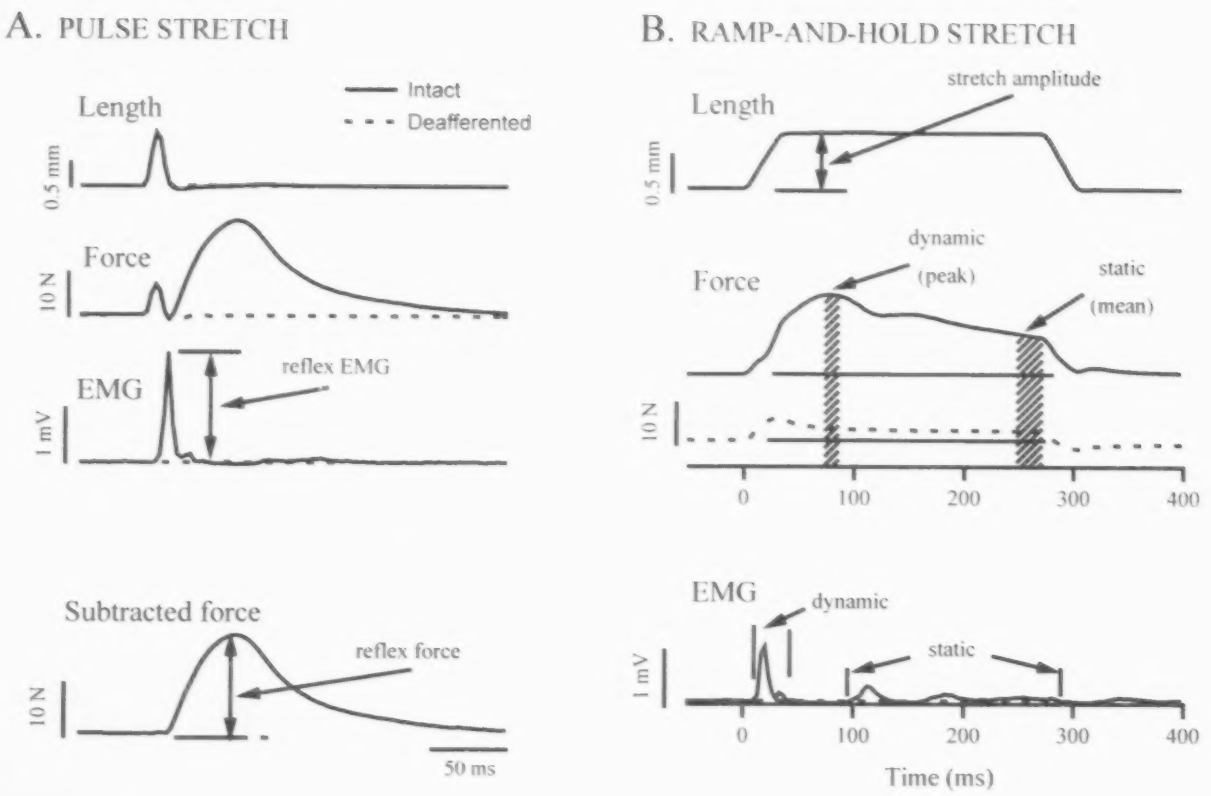

Figure 5.2 - Example of reflex force and stiffness calculations with isometric loading.
A. Reflex response to pulse stretch for both intact (decerebrate, solid line) and deafferented (dotted line) data. The bottom trace represents the reflex force, resulting from the subtraction of the deafferented averaged force from the intact averaged force. B. Reflex response to ramp-and-hold stretch also for both intact and deafferented data. The dynamic force is measured as the peak force in a window ranging from 78 to 84 ms after the onset of the stretch. The static force is measured as the mean force in a window ranging from 250 to 270 ms after the stretch onset. Both measurements are done for intact and deafferented averaged force. Mean EMG is measured for both dynamic and static EMG bursts, as illustrated on the bottom trace.

this EMG burst above the mean background level of activity was measured and computed as the EMG reflex response to stretch.

RAMP-AND-HOLD STRETCH AGAINST ISOMETRIC LOAD. Due to the rather long duration of the ramp-and-hold stretch, a linear trend corresponding to a change (increase or decrease) during tonic or phasic contraction was initially removed from the averaged force signal. The total force was measured on the averaged traces in the decerebrate and spinal conditions for each level of background muscle force at two different latencies in response to the ramp-and-hold stretch (Fig. 5.2*B*): peak force above the mean background force in a window ranging from 78 to 84 ms after the stretch onset (dynamic reflex) and mean force above the mean background force in a window ranging from 250 to 270 ms following the stretch onset (static reflex). Note that a preliminary verification showed that the peak of the dynamic reflex force always occurred in this narrow window (78-84 ms). The dynamic and static total force values were converted into dynamic and static total reflex stiffnesses respectively, by dividing by the stretch amplitude. Similar measurements were performed on the averaged traces in the deafferented condition for the evaluation of the intrinsic (non-reflex mediated) stiffness. The non-reflex mediated stiffness values were fitted with a linear regression line when plotted against the background force. Dynamic and static reflex-mediated stiffnesses were then obtained by subtracting the intrinsic stiffness from the dynamic and static total reflex stiffnesses respectively, at a matched level of background force. For the ramp-and-hold stretches, the force measurements were converted into stiffnesses to allow subtraction of the intrinsic stiffness without the confounding effect of a slightly different amplitude of stretch. As with the pulse stretch, a distinct short-latency EMG volley was present following the onset

of the stretch. This EMG burst (dynamic) was followed by a second EMG volley (static). These two volleys were clearly separated by a silent period. The mean EMG activity was calculated for each of these bursts between window limits which were manually positioned during the analysis, providing dynamic and static reflex EMG responses.

PULSE STRETCH AGAINST COMPLIANT LOAD. The averaged length and force traces recorded in presence of an elastic load were prepared for their match to the computer simulation outputs. Background changes in force and length (linear trend) were removed by selecting two points on each trace (usually one prior to the stretch, and one after completion of the force or length change due to the reflex action). As for the pulse and ramp-and-hold stretches with isometric load, background force and EMG activity were evaluated over a period of 10 and 30 ms respectively prior to the onset of the stretch.

5.2.7 Computer simulations

A simplified model for the mammalian segmental reflex system was used to simulate the experimental results from two cats in isometric as well as in compliant external loading conditions. This dynamic reflex model included among its principal elements: series and parallel elastic components for intrinsic muscle characteristics, an active force element, a transfer function for spindle Ia afferents including a delay and an adjustable gain, a rectifier, an external load which was more or less elastic, and an external perturbation. Some of the model parameters were individually adjusted in order to match as closely as possible the averaged length and force traces for each external load characteristic (isometric, 0.2, 0.5 and 1.5 mm/N), for each experimental conditions (decerebrate tonic, spinal at rest and spinal walking) and at each level of background

force. These parameters were: the external load stiffness, the parallel and series stiffnesses, the gain of the Ia feedback loop, the rate constant for the active force element, and the nominal stretch amplitude to compensate for external load elasticity. Details about how the model parameters were selected are given in APPENDIX A. The modelling procedure described in this paper was performed using MATLAB 4.2c and its associated graphics-based SIMULINK 1.3c control systems software (MathWorks Inc., Natick, MA, USA).

The goodness of the match between the simulated and the experimental data was quantified by the proportion of the variance accounted for (VAF) by the fitted curve. The VAF was calculated for both length and force responses as follows:

$$(1 - \frac{RMS^2}{\sigma^2}) \cdot 100\%$$

where RMS^2 corresponded to the root mean square value quantifying the error between the simulated and experimental traces, and σ^2 was a measure of deviation from the mean (variance) for the experimental data. Model parameters were changed until the sum of the length and force VAF values was optimal.

5.2.8 Reflex loop gain estimation

The gain of the stretch reflex loop was obtained from the computer model set along with the parameters which offered the best fit for each experimental conditions. This gain evaluation was performed by introducing a sinusoidal input to the model (instead of a pulse stretch). The reflex loop gain, obtained for sinusoidal inputs of 1, 2, 5, 7, 10, 14 and

20 Hz, was calculated following a two-step procedure (described in detail in APPENDIX A) similar to the approach used to estimate the reflex loop gain when a mechanical sinusoidal stretch was experimentally applied to the TS muscles (see CHAPTER 4).

5.2.9 Statistical analysis

A variety of statistical tests were performed on the data presented in this paper. Analyses of variance (ANOVAs) or paired-sample t-tests were generally used. Although, when the sets of data did not meet the assumptions of normality and equal variance ($p<0.05$), a nonparametric analysis was conducted with either the Friedman's test or Wilcoxon paired-sample test (Zar, 1984). Furthermore, when the analysis of variance revealed significant differences among multiple sets of data, a multiple comparison procedure such as the Student-Newman-Keuls method was applied in order to determine which of the variances differ from which others. For each statistical result reported below, the test used is indicated in parentheses, along with the level of significance and the sample size. Note that the notation "t-test" refers to a two-tailed paired-sample t-test, unless otherwise specified. All the statistical tests were performed with the commercially available software SIGMASTAT (Jandel Scientific, Corte Madera, CA, USA).

5.3 RESULTS

5.3.1 Effect of muscle background activity

TONIC CONTRACTIONS. In the decerebrate condition, pulse stretches were applied to the TS muscles during tonic contractions produced by the crossed extensor reflex. Since the level of force generated by the crossed extensor reflex varied, it is possible to compare the data for different amount of force developed by the muscles prior to the stretch. In Fig. 5.3*B*, force and EMG traces are shown for both the decerebrate (solid lines) and the deafferented (dotted lines) conditions, for low and high levels of background force. Note the absence of force produced by the reflex response after deafferentation. The reflex force obtained after subtracting the deafferented force responses from those obtained in the decerebrate condition, is also shown for both background activity levels. It is clearly seen in Fig. 5.3*B* that the reflex decreases when more background activity is present in the muscles at the time of application of the stretch. A similar decrease is also noticeable in the short latency reflex EMG burst with a higher level of tonic activity.

PHASIC CONTRACTIONS. After spinalization, locomotion was induced by the administration of Naloxone and Clonidine. Manual perineal stimulation was required to trigger and maintain continuous periods of walking for 1-2 minutes. Only cycles where the animal was walking regularly were analyzed and the responses to stretch occurring at the same time in the step cycle were averaged together. Similar to the data obtained during tonic contractions, the level of background force present in the muscles at the time of application of the stretch was measured. Fig. 5.3*C* represents averaged data for low

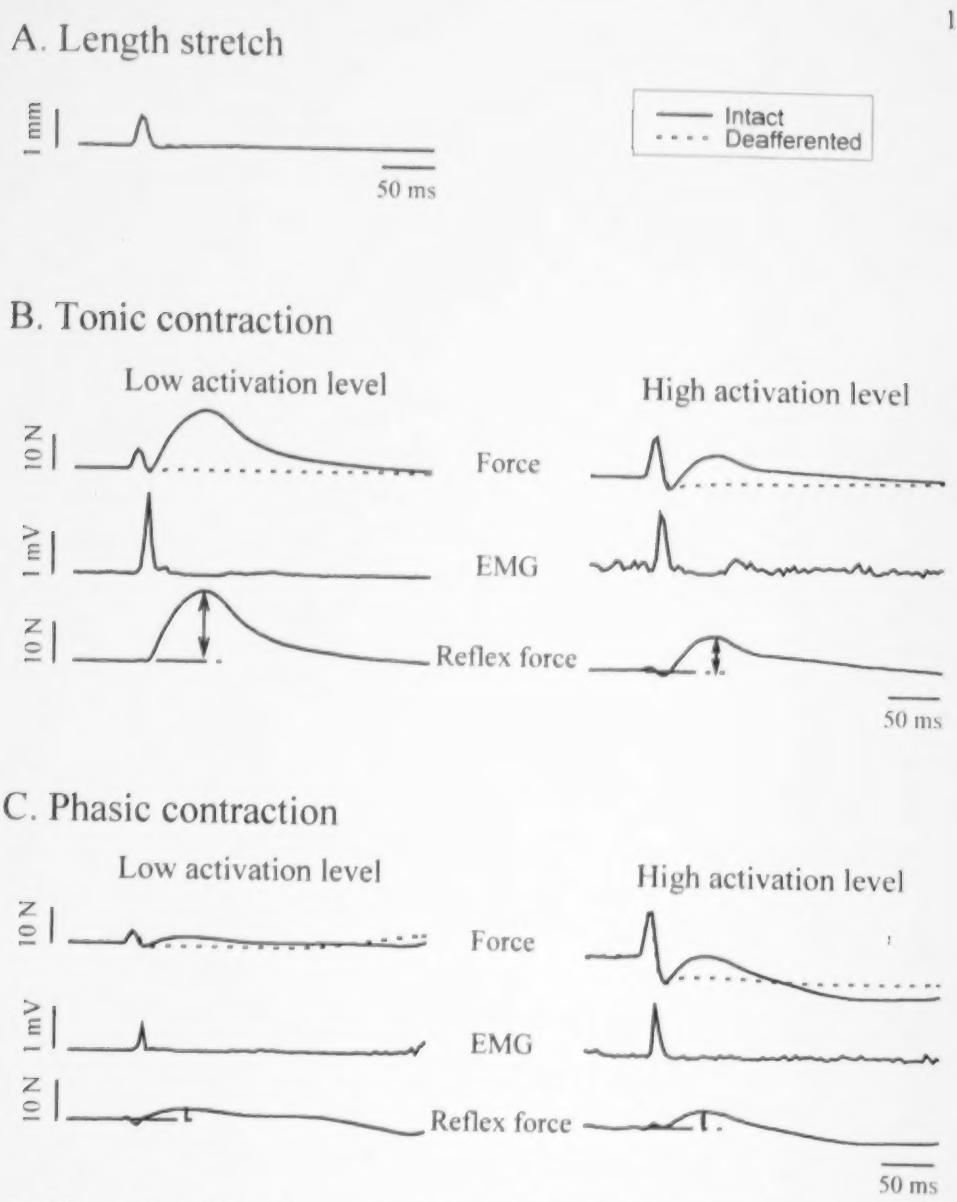

Figure 5.3 - Typical reflex responses to pulse stretch during tonic and phasic contractions.

A. Averaged length trace representing a pulse stretch in isometric condition. *B*. Averaged total force, EMG and reflex force in response to a pulse stretch, for low (left panel) and high (right panel) level of background force, in tonically active TS muscles (decerebrate). Remember that the reflex force is obtained by subtracting the averaged force recorded after deafferentation (dotted line) from the averaged force obtained with intact dorsal roots (solid line, see also Fig. 5.2). Arrows indicate the reflex force measurement. *C*. Averaged total force, EMG and reflex force in response to pulse stretch, for low (left panel) and high (right panel) level of background force, in phasically active TS muscles (spinal walking). Same format as in *B*.

and high level of background force during phasic contractions (locomotion). In addition to the fact that the reflex seems highly attenuated during walking when compared to the postural task, the behaviour of the reflex force in relation to the background force is opposite to the one seen during tonic contractions. The reflex force was rather small when there was almost no activity in the muscles (corresponding to the flexion phase of the walking cycle), and augmented with an increase in the background force (corresponding to the extension phase of the walking cycle). The TS EMG reflex burst also increased when the background activity was greater.

The modulations in the reflex force over a full range of background force are clearly represented by the results from one cat in Fig. 5.4 (top graph). In the decerebrate condition, the reflex force decreased when the background force was augmented by tonically contracting the muscle. In the spinal condition, an opposite relationship is seen where the reflex force increased when the background force was augmented by phasically activating the muscle to produce locomotion. Similar trends can be observed in the reflex EMG (Fig. 5.4, bottom graph) but the relationships are not as distinct as for the reflex force data.

Spinal cats treated with Clonidine display intermittent periods of walking, triggered and maintained by manual perineal stimulation. This peculiarity allowed us to stretch the TS muscles between locomoting intervals while the muscle was at rest. In this particular condition, the reflex force was attenuated when compared to the decerebrate data for a similar level of background force, while remaining greater than the reflex response measured during locomotion (open circle in Fig. 5.4).

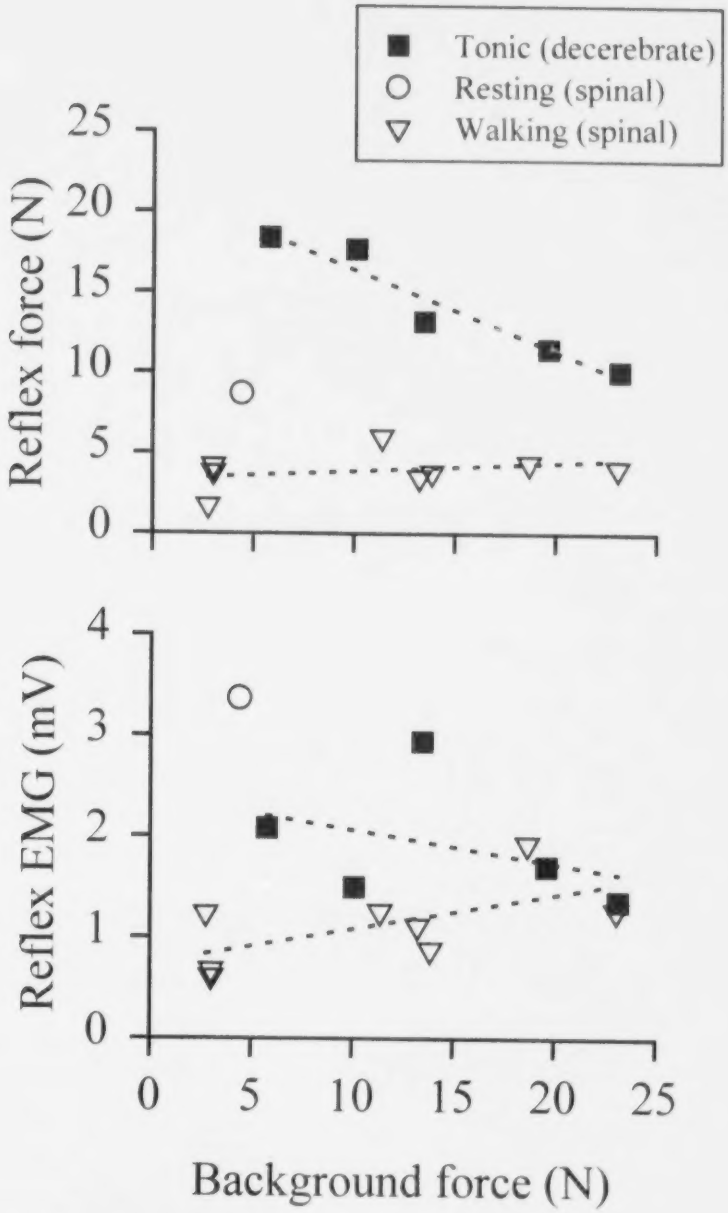

Figure 5.4 - Reflex responses across background force levels and across tasks. Reflex force (top graph) and EMG (bottom graph) plotted against the background force levels in tonically active muscles (filled squares) and phasically active muscles (open triangles) for one cat in isometric conditions. Data acquired at rest, after spinalization and administration of Clonidine are also indicated (open circle). Note the opposite relationship between reflex and background forces when comparing tonic contractions with phasic contractions (walking).

Due to the wide variation in the ranges of background forces generated by the TS muscles between the seven cats studied in the current series of experiments, the reflex data were normalized using the following steps: (1) the data were grouped in three distinct levels of background force (low, moderate and high), and (2) the reflex force and EMG were expressed as percentages of the responses measured in the spinal cat at rest. The normalized results are shown in Fig. 5.5 and confirmed the opposite relationships between reflex and background forces observed during tonic and phasic contractions. Note that the normalized results include the data from both 1 and 3 mm stretch amplitudes, differences between the two stretch amplitudes were shown to be not significant ($p>0.1$). Effectively, the reflex force significantly decreased with increasing tonic contractions in the decerebrate condition while the same reflex force significantly augmented with increasing phasic contractions in the spinal condition (one way ANOVA with repeated measures, $p<0.05$, $n=10$). A pairwise multiple comparison test specificd that the reflex force for low tonic contractions was significantly greater than for moderate and high tonic contractions (Student-Newman-Keuls method, $p<0.02$). A similar test applied to the spinal walking data pointed out a significant difference only when comparing the low and the high levels of background contractions ($p<0.02$).

At low levels of background contraction, the reflex force was larger in the decerebrate cat during tonic contraction than in the spinal cat during walking (two way ANOVA with repeated measures, $p<0.05$, $n=10$). Two experimental factors may have caused this decrease in the stretch reflex. First, the spinalization performed acutely provoked a spinal shock and could affect the stretch reflex. Second, Clonidine has been shown to reduce fusimotor drive (see CHAPTER 3), consequently decreasing the spindle

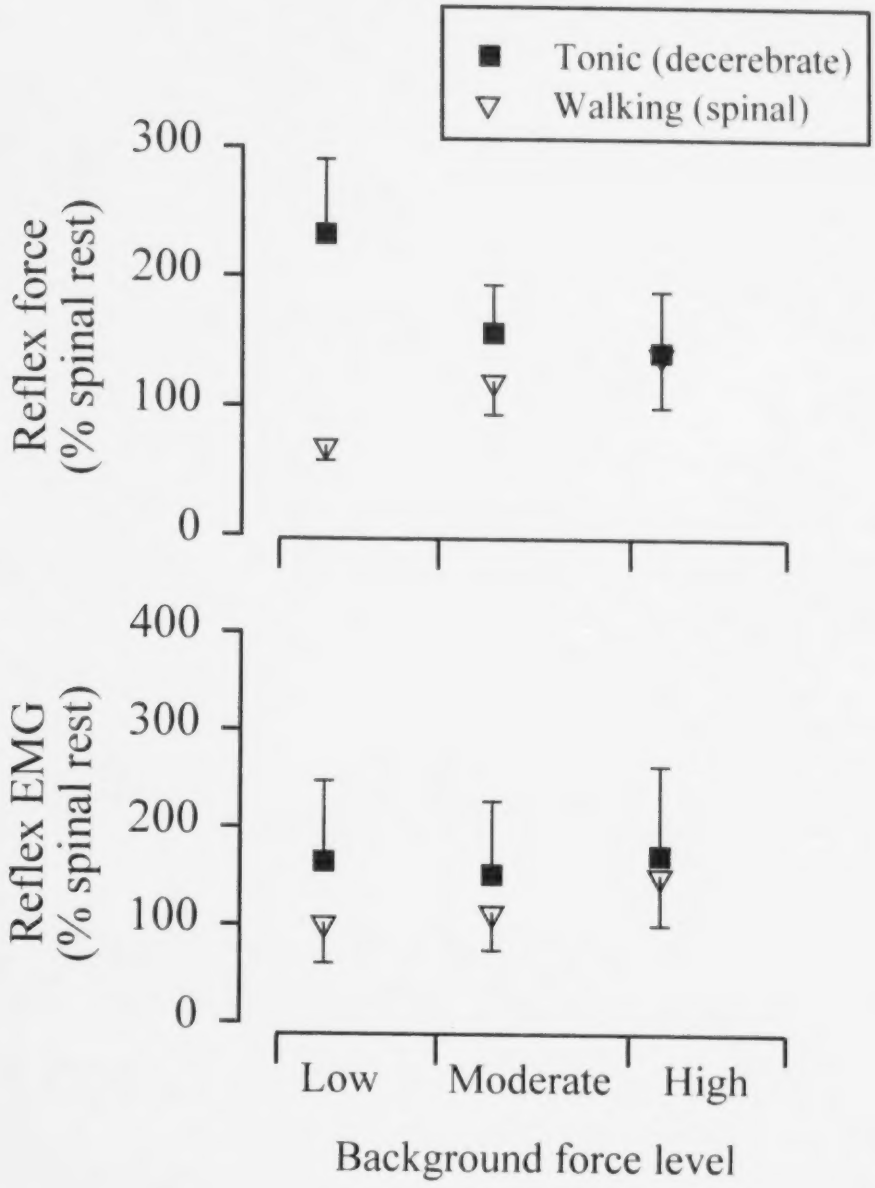

Figure 5.5 - Normalized and ensemble reflex responses across background force levels and across tasks.

Reflex force (top graph) and EMG (bottom graph) plotted against the background force levels for both types of contractions, after normalization. The data for all cats (similar to the data presented in Fig. 5.4), are grouped in three distinct levels of background force (low, moderate and high), and the reflex force and EMG are expressed as percentages of the responses measured in the spinal state at rest. Each point represents a mean value of reflex force or EMG with its standard error.

sensitivity to stretch. A statistical analysis using the data obtained in the spinal cat at rest proved that these two factors cannot solely account for the observed drop in the reflex force in the spinal walking cat. The reflex force was significantly smaller during walking than during the resting condition in the spinal cat (one-tailed paired-sample t-test, $p<0.01$, $n=10$). Remember that the resting periods were interspersed between periods of locomotion. Therefore the data obtained in the spinal cat at rest was also acquired after the administration of both Naloxone and Clonidine. No significant differences were found in the reflex EMG responses between tonic and phasic contractions, at all levels of background force (two-way ANOVA with repeated measures, $p>0.05$, $n=10$).

5.3.2 Ramp-and-hold stretch

Only short-latency reflexes were elicited with the pulse stretch. The use of a ramp-and-hold stretch, including a plateau phase of 240 ms, evoked a longer latency reflex in addition to the short-latency reflex. The first and second EMG reflex volleys produced changes in force which were measured and converted into dynamic and static stiffnesses respectively. Typical relationships of total, intrinsic and reflex-mediated stiffnesses with tonic background muscle force are illustrated in Fig. 5.6, for a 1 mm ramp-and-hold stretch. Similarly to the results obtained with the pulse stretch in the decerebrate condition, the reflex-mediated stiffness due to the first burst of reflex EMG (dynamic stiffness) decreased with increasing tonic muscle activity (Friedman, $p<0.05$, $n=12$). A pairwise multiple comparison test specified that the dynamic stiffness for low and moderate levels of background force were significantly greater than for high force level (Student-Newman-Keuls method, $p<0.05$, $n=12$). On the other hand, the static

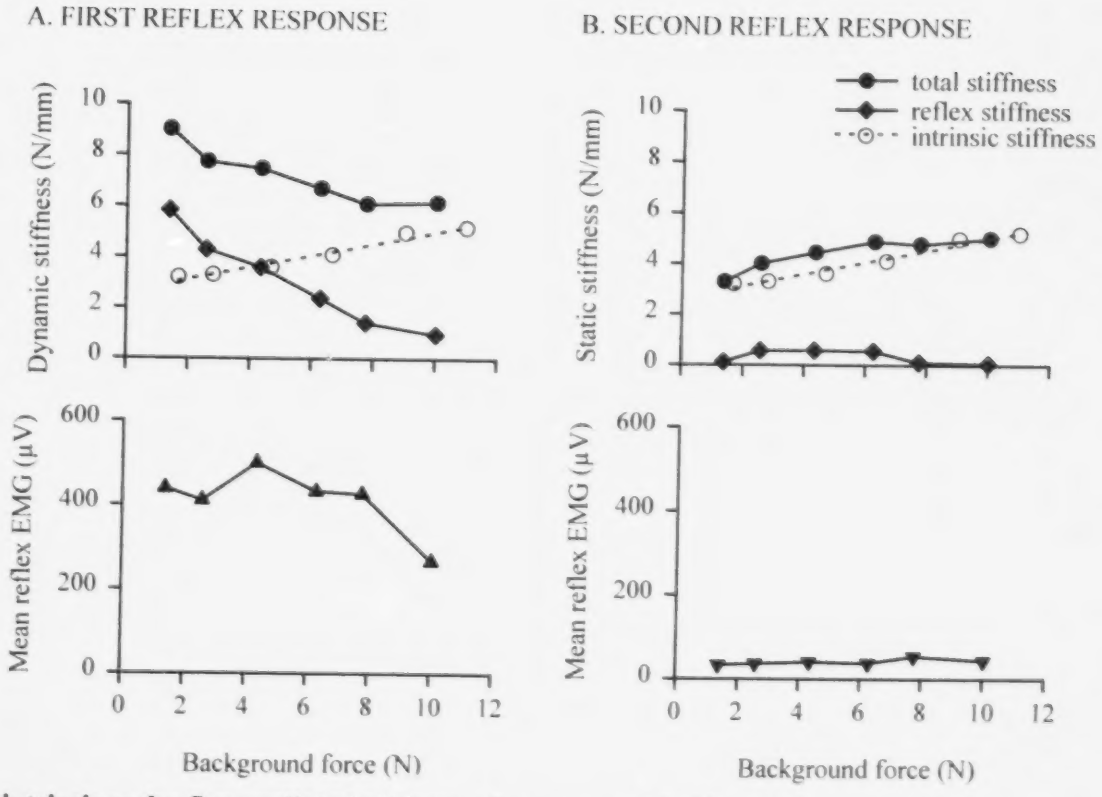

Figure 5.6 - Total, intrinsic and reflex-mediated stiffnesses for ramp-and-hold stretch.
Total, intrinsic and reflex-mediated stiffnesses as measured with a ramp-and-hold stretch in a decerebrate cat, plotted against the level of background force produced by a crossed-extensor reflex, with isometric loading. *A*. Dynamic stiffness due to the short-latency reflex (top graph) and reflex EMG (bottom graph). The reflex-mediated stiffness (filled diamonds) is obtained from the subtraction of the intrinsic stiffness (open circles, regression shown as dotted line is used to estimate the intrinsic stiffness at a matched level of force of the total stiffness data) from the total stiffness (filled circles). *B*. Static stiffness due to long-latency reflex (top graph) and reflex EMG (bottom graph). Same format as in *A*.

stiffness (due to the second burst of reflex EMG) was much smaller when compared to the dynamic stiffness and presented a different relation when plotted against background force (Fig. 5.6*B*): it gradually increased for low background forces, peaked in the middle of the range and declined toward zero for higher force levels. These variations in the static stiffness were not significant (Friedman, $p>0.1$, $n=12$).

For completing the statistical analysis, the above results were grouped into low, moderate and high levels of background activity as for the pulse stretch data. Preliminary tests indicated a small but significant decrease in dynamic stiffness from 1 to 3 mm stretch (Wilcoxon paired-sample test, $p<0.02$, $n=6$) whereas no significant differences were detected for the static stiffness between the two stretch amplitudes (t-test, $p>0.8$, $n=6$), after verification that these two sets of data were taken at matched levels of background force (t-test, $p>0.7$, $n=6$). Since the variations across force levels were comparable in both stretch amplitude data sets (for either dynamic or static stiffness), data for 1 and 3 mm stretches were combined for the final statistical tests presented in the previous paragraph.

For a ramp-and-hold stretch, the EMG response typically consisted of two bursts: a dynamic response with an onset latency of 8 ± 2.8 ms (mean ± SE, short-latency response) and a static response with an onset latency of 83 ± 12.7 ms (mean ± SE, long-latency response). As indicated in Table 5.1, the latency of the static response decreased from 92.7 ms at low force levels to 75.3 ms when the background force was high (Friedman, $p<0.005$, $n=12$). A pairwise multiple comparison test confirmed a significant decrease in the latency of the static response between each level of background force (Student-Newman-Keuls, $p<0.05$, $n=12$). On the other hand, the latency variations for the

dynamic response were not significant. The fact that motor units are firing synchronously during the second burst of EMG, and that their firing rate is greater as the background muscle force increases might explain this decrease in the latency. Since there was no significant difference in the reflex latencies for 1 and 3 mm stretches (t-test, $p>0.05$, $n=18$), data from both stretches were combined for the statistical analysis.

	BACKGROUND FORCE LEVELS		
	LOW	MODERATE	HIGH
PULSE STRETCH			
short-latency reflex	8.7 ± 1.9	8.3 ± 1.9	9.0 ± 2.4
RAMP-AND-HOLD STRETCH			
short-latency reflex	8.6 ± 1.7	7.1 ± 3.3	8.3 ± 3.2
longer latency reflex	92.7 ± 13.9	81.8 ± 9.4	75.3 ± 7.9

Table 5.1 - EMG latencies for pulse and ramp-and-hold stretches.
(mean±SD in ms, n=10 for pulse stretch, n=12 for ramp-and-hold stretch).

The mean amplitude of the dynamic EMG reflex response showed a slight decrease (although non significant, Friedman, $p>0.1$, $n=12$) with increasing background contraction level while the mean amplitude of the static reflex response was invariant throughout the range of forces produced by the tonic contraction (Friedman, $p>0.3$, $n=12$). Note that the static mean EMG responses were substantially smaller than the dynamic responses (t-test, $p<0.001$, $n=18$). Further analyses of the EMG data revealed larger short-latency EMG responses for a 3 mm stretch when compared to the 1 mm stretch, at all levels of

background activity (t-test, $p<0.05$, $n=6$). On the other hand, the second EMG reflex volley was comparable for both 1 and 3 mm ramp-and-hold stretches (t-test, $p>0.1$, $n=6$), except at low level of background activity where a larger response was seen with a 3 mm stretch (t-test, $p<0.01$, $n=6$).

5.3 3 Effect of external load stiffness

In the course of these experiments, the motor used to stretch the muscles was set to act as an elastic load of various compliances: isometric, 0.2, 0.5 and 1.5 mm/N (the compliance being equal to the inverse of the stiffness). The last three numbers mean that for each Newton of force developed by the muscle during a contraction, the manipulandum was moving by 0.2, 0.5 and 1.5 mm respectively. The effect of this variation on the stiffness of the motor is shown in Fig. 5.7. Averaged length and force for one cat in the decerebrate condition are presented starting with the isometric condition (top traces) to gradually increasing load compliance towards the bottom of the figure. The reflex change in length clearly became much larger as the compliance augmented, whereas the reflex force became smaller. A more detailed analysis of these results will be presented in the next section, along with the computer simulation results. Note that with weaker loads the shortening was substantial and could be more than twice the amplitude of the stretch (e.g. see 1.5 mm/N length trace in Fig. 5.7).

5.3.4 Computer simulations

Computer simulations, using a simplified model of the mammalian stretch reflex loop, were performed to match the experimental data acquired with isometric and non-

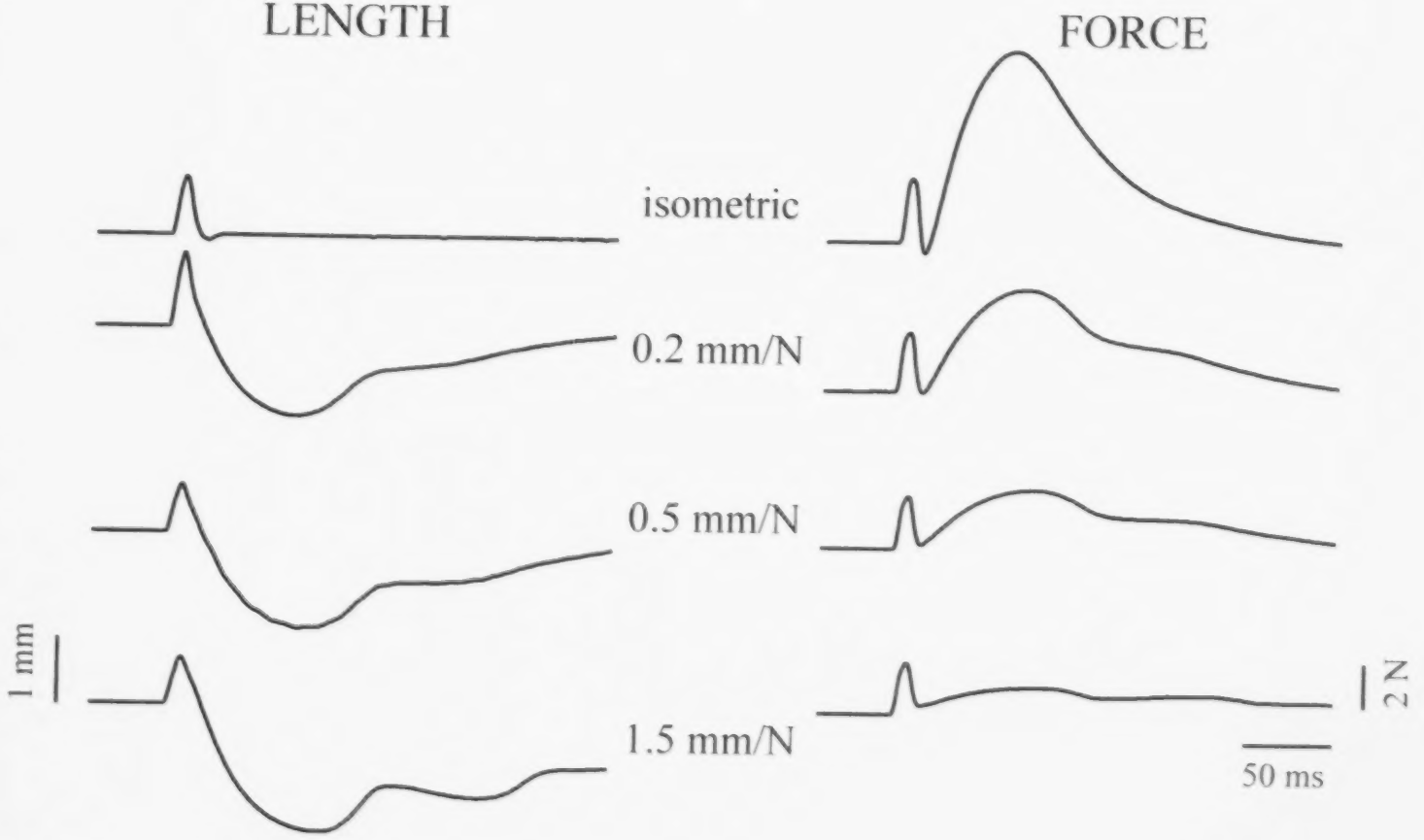

Figure 5.7 - Examples of effect of compliant external load on reflex response to pulse stretch.
Length (left pannel) and force (right pannel) responses to pulse stretch for isometric and compliant external loads (0.2, 0.5 and 1.5 mm/N). Note the increase in muscle shortening with a more compliant load accompanied by a decrease in reflex force.

isometric external loads. Examples of experimental and simulated traces, in the decerebrate, spinal at rest and spinal walking conditions are illustrated in Fig. 5.8. In these examples, the external load was elastic (0.2 mm/N) and the background level of muscle activation was moderate. As mentioned earlier, variance accounted for values (VAF, specified in brackets) were calculated as an indicator of the goodness of fit. Note that the VAF values are very close to 100%, indicating a very good although not perfect match between the experimental and the simulated data. Also, note the change in the time course of the decline in reflex force which increased from decerebrate to spinal at rest and from spinal at rest to spinal walking. This last observation was supported by examining the changes in the rate constant selected when fitting the simulations to the experimental data for these different conditions (corresponding to the decay rate of the active state). This rate constant was smaller for the spinal data (both at rest and during locomotion) when compared to the decerebrate condition, giving rise to a prolonged time course in the reflex force decay in the latter condition (Fig. 5.9). Since the muscles studied include the soleus muscle (mainly composed of slow twitch fibres) and the gastrocnemii muscles (with a mixed composition of slow and fast twitch fibres), it is possible that the motor unit recruitment among these muscles varied between the tasks (for both background and reflex forces), which may account for the differences seen in the rate constant.

Also, this active force rate constant increased when the external load became more compliant and presented a positive relationship with the tonic background activity (Fig. 5.9*A*). This increase in the rate constant for more elastic loads had also been observed for twitch responses in the cat plantaris muscle (see Fig. 6 in Bawa *et al.* 1976a).

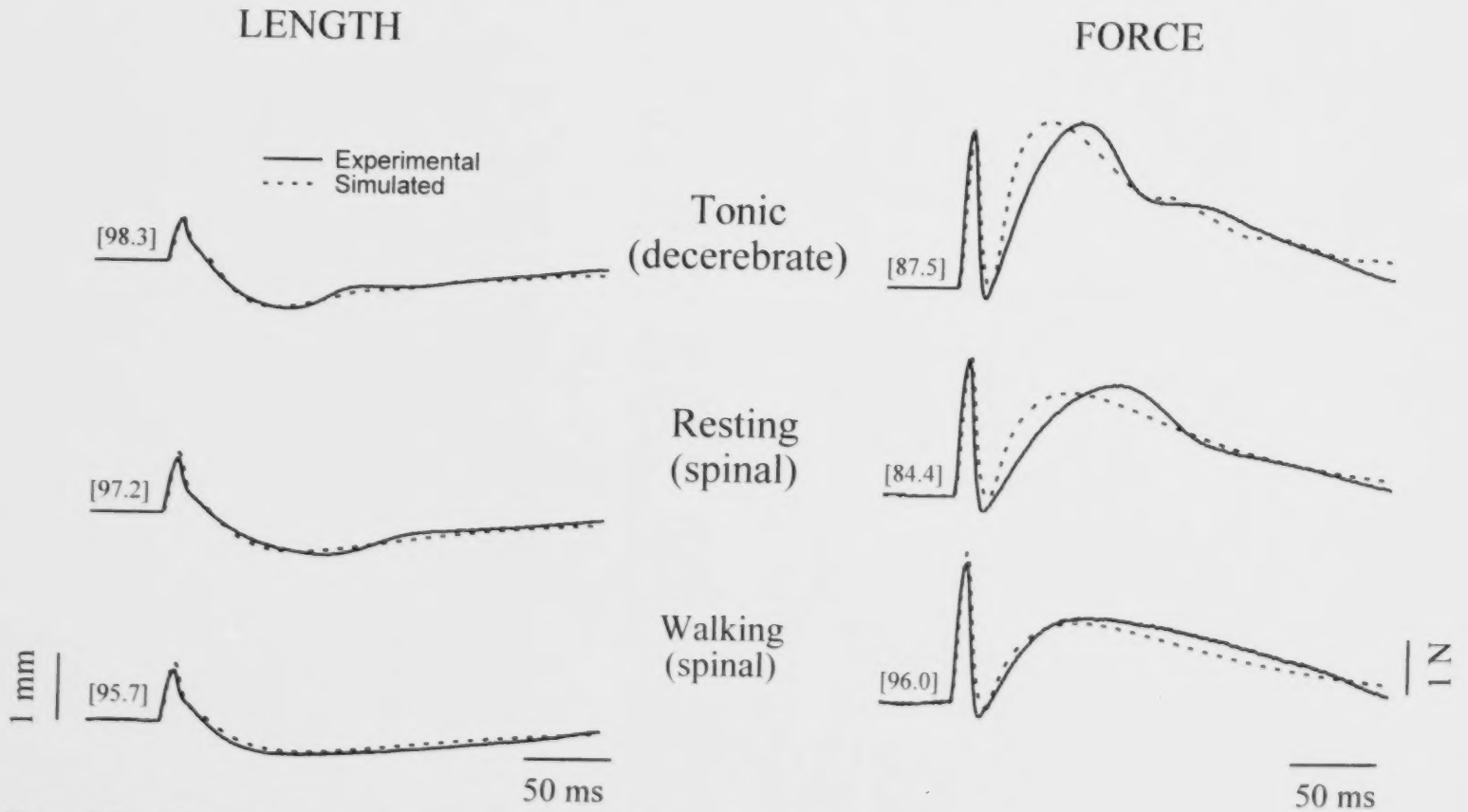

Figure 5.8 - Examples of experimental and simulated data across tasks.
Simulated responses to a pulse stretch superimposed to the averaged length (left pannel) and force (right pannel) obtained experimentally during tonic contractions, and in a resting and walking spinal cat. The numbers in brackets indicate the proportion of the variance accounted for (VAF) by the fit, calculated for verification of goodness of fit. Experimental data is from one cat with an external load compliance of 0.2 mm/N.

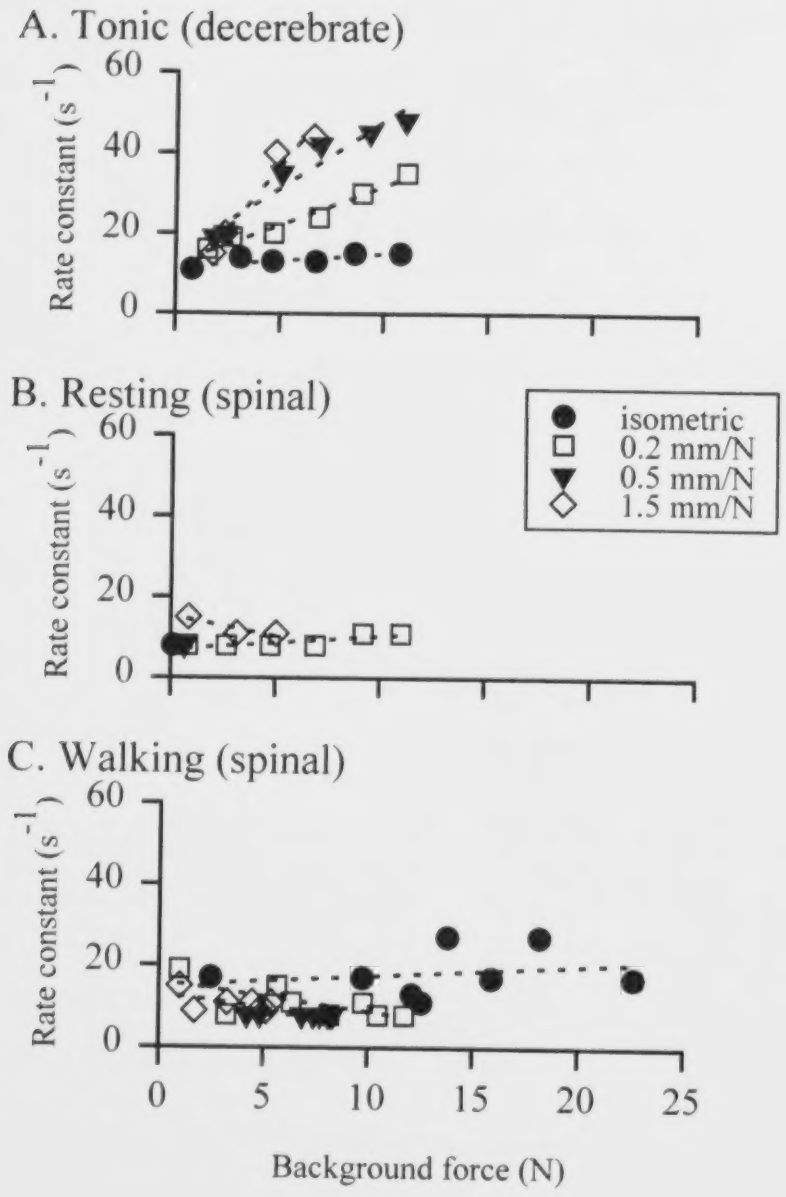

Figure 5.9 - Rate constant variations across external load compliances and across tasks.

Rate constant of the transfer function of the muscle contractile elements of the model, as selected to fit the simulations to the experimental data (pulse stretch). The rate constant is plotted against the background force for isometric and compliant loads (0.2, 0.5 and 1.5 mm/N) for each task: during tonic contraction (*A*), during resting or tonic contraction after spinalization (*B*) and during locomotion (*C*). Note the gradual increase in the rate constant with an increased background force, during tonic contractions. Data from one cat.

5.3.5 Gain of the stretch reflex loop

ISOMETRIC LOADING. The gain of the stretch reflex loop was estimated using the model, with the parameters selected for each experimental condition studied in two cats. Results of gain magnitude and phase amplitude with an isometric external load for tonically contracting muscles in the decerebrate state, as well as for resting and walking data in the spinal cat are presented in Fig. 5.10. These results are shown for a range of background forces in tonically and phasically active triceps surae muscles. The reflex loop gain is greater during tonic contraction when compared to walking. As for the reflex force (Fig. 5.4), the gain decreases when the background force is augmented by tonically contracting the muscle (greater than 5 at low background force to less than 1 with higher muscle activity). In the spinal condition, the gain is rather constant and is much smaller than for the tonic contractions (generally less than 1), especially at low levels of background force. Note that the gain in the spinal condition at rest is lower than in the decerebrate state but remains higher than during walking. The results shown in Fig. 5.10 are for a 2 Hz sinusoidal stretch in one cat. Very similar results were obtained from both cats studied with the simulations.

Along with the gain estimate, the phase amplitude of the reflex loop was calculated for each condition. Due to delays in the reflex loop, the output came with a phase lag ranging from -2^0 to -32^0 (Fig. 5.10, bottom graph), being very similar for decerebrate tonic and spinal walking conditions. These results of gain magnitude and phase amplitude presented similar variations than the numbers obtained by experimentally stretching the triceps surae muscles with a sinusoidal waveform (see Fig. 4.7 in CHAPTER 4). Both showed a high gain at low levels of background force during tonic contractions, gain

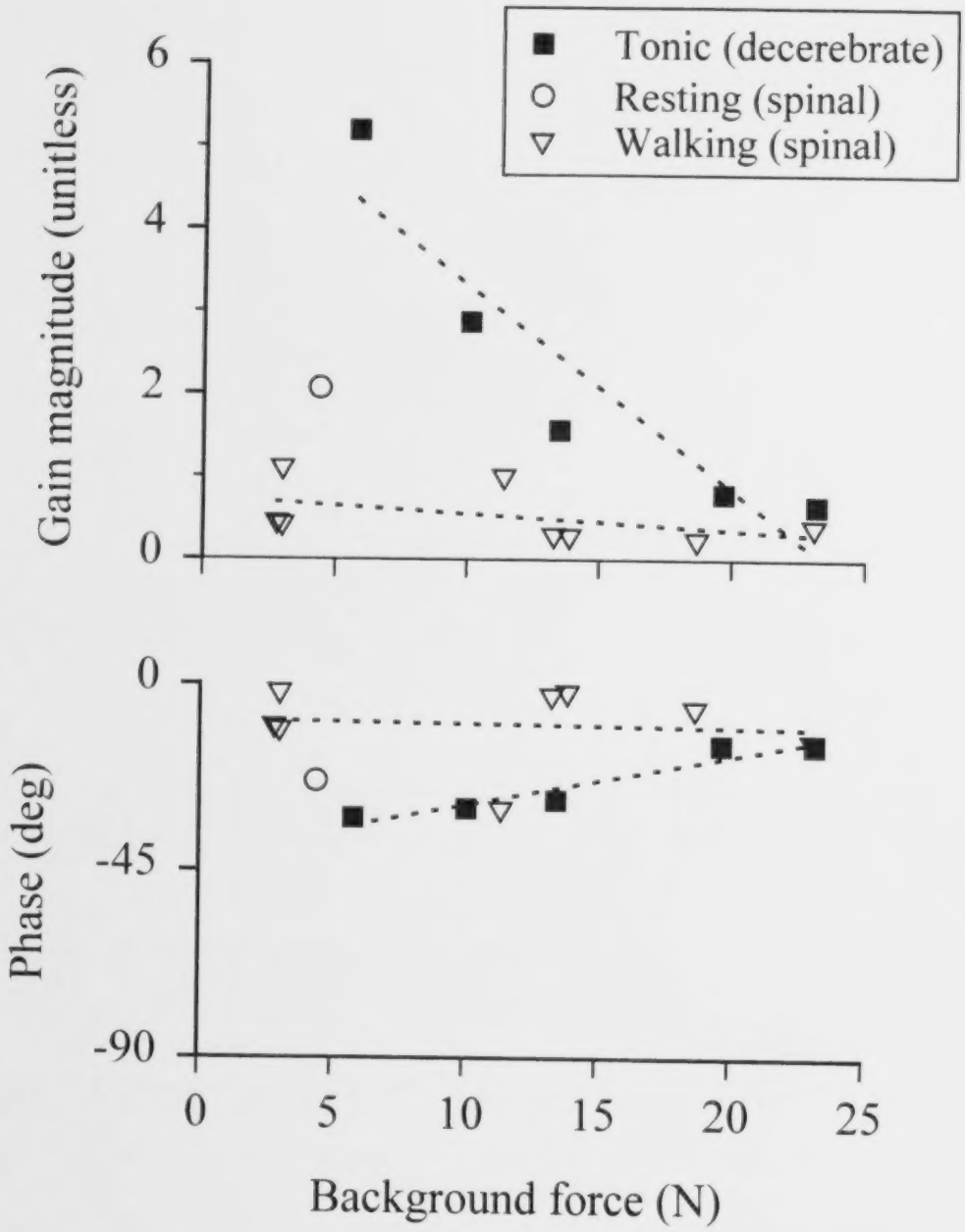

Figure 5.10 - Gain magnitude and phase of the stretch reflex loop with isometric loading.

Reflex gain magnitude (top graph) and phase (bottom graph) is plotted against background force for tonic, resting and walking conditions. The gain was calculated by feeding a sinusoidal input (2 Hz) to the model. See details in APPENDIX A. Note that the relationships between gain and background force for all tasks are similar to those observed between reflex and background forces (see Fig. 5.4). Data from one cat.

which was decreasing and comparable to the gain in the walking spinal preparation when higher levels of muscle activation were attained. However, the gain magnitudes estimated with the pulse stretch were much higher than those obtained with a sinusoidal stretch directly applied to the triceps surae muscles, for the same cat preparations. Reasons for such high values of loop gains will be considered in the DISCUSSION. Note that the simulations reported here were run with two cats in the present study, one of which had also been subjected to the sinusoidal stretches (see CHAPTER 4).

ELASTIC LOADING. The gain of the reflex loop was also calculated for the data acquired when the external load acted as a spring. For all three experimental conditions (decerebrate tonic, spinal at rest and spinal walking), gain magnitudes and phase amplitudes are presented in Fig. 5.11 for external load compliances of 0.2, 0.5 and 1.5 mm/N. Similar to the result obtained with the isometric loading, the gain was greater during tonic contractions than during walking, especially at low levels of background force. Also, the magnitude of the reflex loop gain increased with more compliant external load, mainly for low levels of background force. No clear difference could be seen with higher levels of tonic activity in the triceps surae muscles, or during walking. The rather small magnitude of the loop gain during locomotion prevented a distinct variation across external load compliances. Furthermore, the phase lag of the reflex feedback loop was independent of both the external load stiffness (Fig. 5.11, bottom graphs) and the level of muscle activation (for both tonic and phasic muscle contractions).

Fig. 5.12 presents the variation of the loop gain across frequencies for all three elastic loads at a low level of background muscle activation (generally less than 3 N) in one cat. In all cases, for tonic contractions, the gain is rather high for low frequency and

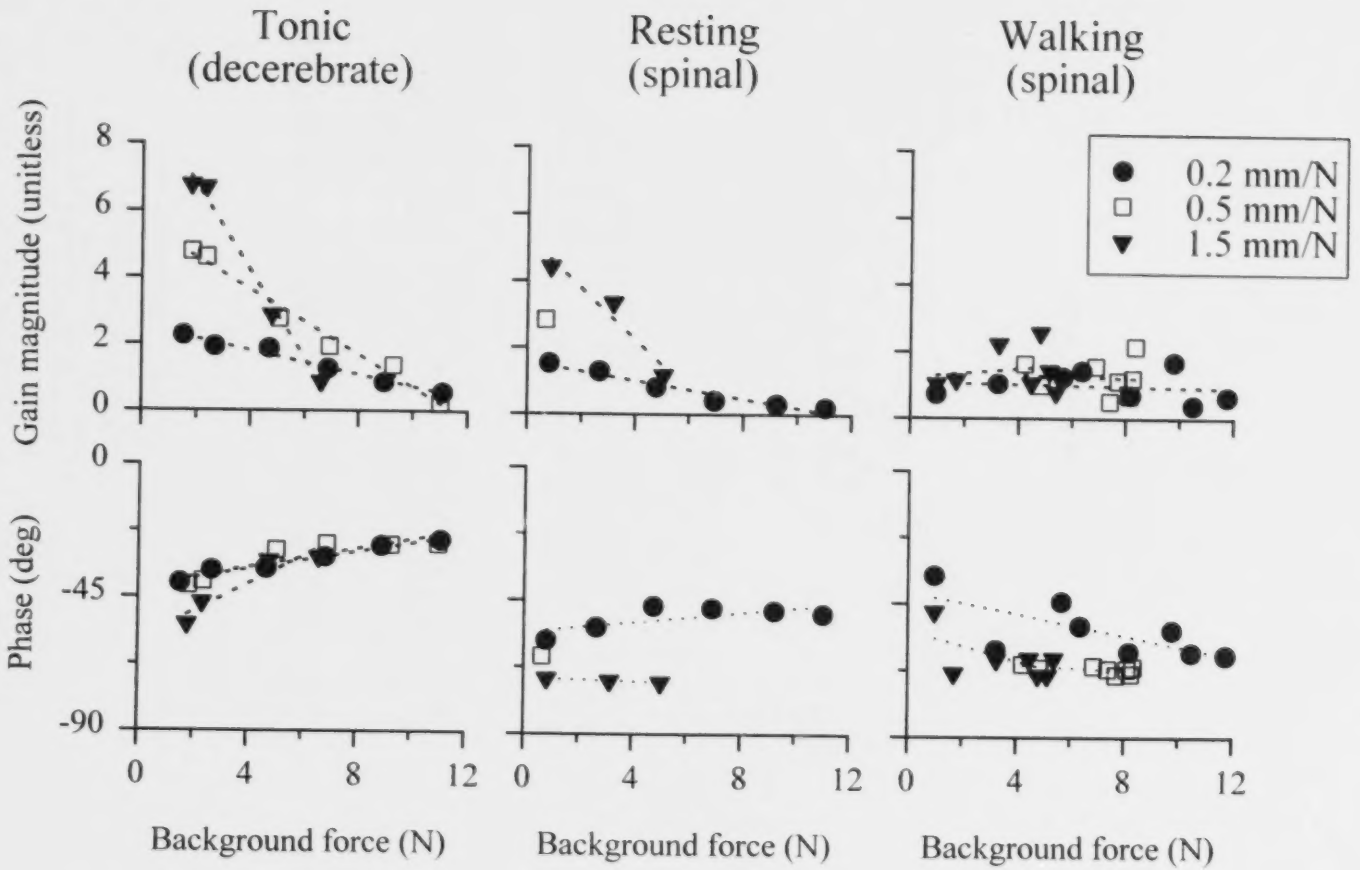

Figure 5.11 - Reflex loop gain magnitude and phase across background forces for non isometric loading.
Reflex gain magnitude (top graphs) and phase (bottom graphs) for all tasks for compliant loads of 0.2, 0.5, and 1.5 mm/N, plotted against the background force. The gain was estimated by feeding a 2 Hz sinusoidal stretch to the model (see details in APPENDIX A). Data from one cat.

gradually decreases to values smaller than 1 as the frequency increases. The magnitude of the reflex loop gain is greater with more compliant external loads and this difference seems to be more pronounced at low frequencies (up to 7 Hz). During locomotion, the gain is generally smaller than 1 throughout the range of frequencies and also decreases with increasing frequency. This decrease in gain with frequency is accompanied by an increase in the phase lag to up to 270^0. An interpolation of the frequency featuring a phase lag of 180^0 was performed for each set of data presented in Fig. 5.12. During tonic contractions, the gain at the interpolated frequency is smaller than 1 only for the stiffer load (0.2 mm/N), suggesting that the system should have become unstable when the compliance of the external load increased for a perturbation at the interpolated frequency.

5.4 DISCUSSION

The results of the present study can be summarized as follows: (1) even in reduced preparations, there is a task-dependent modulation of the stretch reflex when comparing tonic and phasic muscle activation, (2) dynamic and static stretch reflexes present a different behaviour when plotted against background force, which suggests a specific role for each of them in the regulation of joint stiffness, (3) the gain of the stretch reflex loop is large when measured with pulsatile stretches when compared to more continuous perturbations such as sinusoidal stretches, and (4) the reflex loop gain calculated during tonic contractions increases when the external load becomes more compliant while remaining mostly unaffected by the load properties during walking.

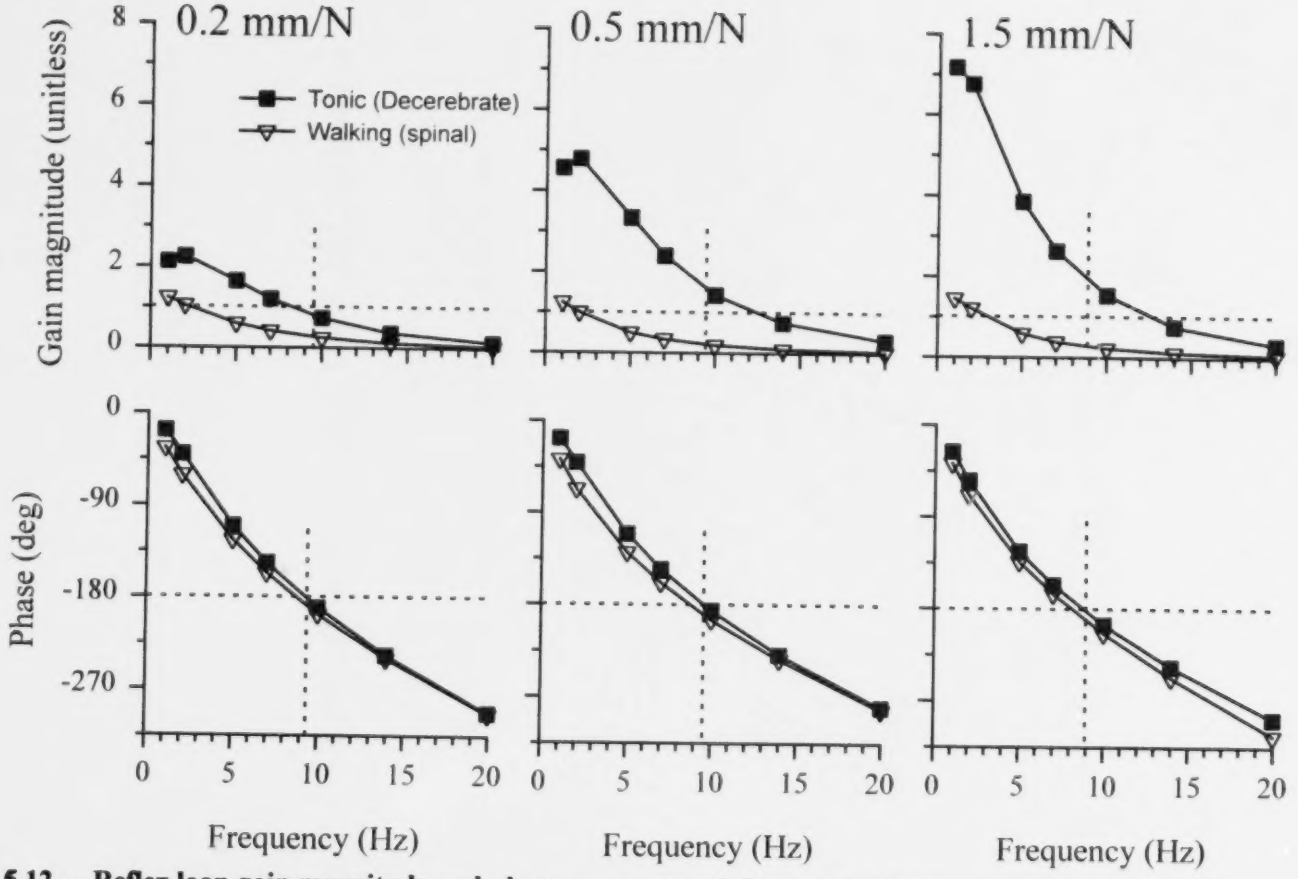

Figure 5.12 - Reflex loop gain magnitude and phase across stretch frequencies for non isometric loading.
Reflex gain magnitude (top graphs) and phase (bottom graphs) measured during tonic and phasic contractions, for compliant loads of 0.2, 0.5, and 1.5 mm/N, plotted against the stretch frequency. Vertical dotted lines indicate the frequency at which the phase lag reaches 180^0 for each load. Note that at this specific frequency the gain magnitude is below 1 for the 0.2 mm/N load while it is above 1 for 0.5 and 1.5 mm/N loads, during tonic contractions. Data from one cat.

5.4.1 Task-dependent reflex modulation

The reflex response to a pulse stretch was modulated when applied during locomotion. The reflex force increased when the background activity was augmented (during the extension phase of the step cycle) and was small when the muscle was quiet (during the flexor phase). This modulation in the stretch reflex matches the variation seen by Akazawa *et al.* (1982) in decerebrate cats. The fact that the reflex modulation during walking remains after spinalization suggests that it occurs at the spinal level. This modulation also compares to the variations observed in the H-reflex, during walking in humans (Capaday & Stein, 1986; Crenna & Frigo, 1987; Yang & Whelan, 1993) and in decerebrate cats (Akazawa *et al.* 1982).

This similarity between stretch and H reflex behaviours refutes the possibility that a cyclic variation in fusimotor activity or afferent volley may account for the observed modulation. Instead, changes in presynaptic inhibition to α-motoneurones are likely to explain the modulation. Indeed, several studies have shown that presynaptic inhibition plays a role in the modulation of the H-reflex during walking (Capaday & Stein, 1987, 1989; Edamura *et al.* 1991; Yang & Whelan, 1993). Presynaptic inhibition may also be responsible for reducing the stretch reflex response during walking when compared to tonically active muscle. However, the sensitivity of Ia afferent spindle receptors presented a marked decrease at the onset of locomotion (see CHAPTER 3), most likely caused by a decrease in dynamic γ-drive combined with a substantial increase in static γ-drive. The change in sensitivity may also be involved in the difference in the stretch reflex between tonic and phasic contractions.

The reflex force recorded from a pulse stretch during crossed extensor reflex (tonic muscle activation) was not only larger than during locomotion but also displayed a reverse relationship when plotted against background muscle force. This opposite relationship between reflex and background forces seen when comparing tonically and phasically active muscles suggest that different control mechanisms are regulating the size of the reflex response in the two tasks. In human H-reflex studies, it was shown that the relationships between reflex size and muscle background activity were in the same direction (although different slopes) when comparing walking with standing. This discrepancy between stretch and H responses could be explained by a distinct fusimotor drive to control afferent receptor sensitivity. In fact, results obtained from direct recordings of Ia afferents in the cat soleus muscle suggested that the static γ-drive is increased during crossed extensor reflex while dynamic γ-drive remains unchanged (when compared to relax state, see Table 3.3 in CHAPTER 3). This rather constant dynamic γ-drive during tonic activity could partly be responsible for the larger reflex responses seen in tonically active muscles, when compared to locomotion where dynamic γ-drive was decreased.

Another aspect of the monosynaptic reflex loop pathway is neglected in the H-reflex studies: the amount of force produced by the reflex volley. Conclusions are usually drawn based on the peak-to-peak size of the EMG response, and not on the amount of force resulting from the neural reflex volley. In humans, it was demonstrated that the twitch force generated by a H-reflex was proportional to the H response amplitude but the slope of the relationship was changing depending on the amount of background activity (Gottlieb & Agarwal, 1971). Incidentally, for the same H-reflex peak-to-peak amplitude,

the twitch force was smaller when the muscle was already contracted when compared to the relaxed condition. Gottlieb and Agarwal (1971) proposed that for a reflex elicited in a muscle which is voluntarily contracting, the twitch is reduced because the two activating signals summate within the same motor units rather than solely by recruiting additional ones. This gradual decrease in the twitch force produced by an H-reflex elicited in an already contracting cat soleus muscle was also observed in recent experiments (S.J. De Serres, J. E. Misiaszek, R.B. Stein & K.G. Pearson, unpublished observations). The absence of a net decrease in the reflex EMG at higher background forces, as seen in the current study, provides further support for this hypothesis.

Finally, the sarcomere 'give' theory, presented with frog (Flitney & Hirst, 1978) and cat muscles (Rack & Westbury, 1974) may explain the decrease in reflex force seen with augmenting background levels of muscle activation. This theory relies on the fact that more bonds between the thick and thin filaments are broken by the stretch at higher forces as compared to the low force levels. The effect of the reflex is probably just able to replace these broken bonds and to bring the tension back to its initial level.

5.4.2 Role of the stretch reflex in stiffness regulation

In the current study, two types of perturbation were used to stretch the TS muscles in decerebrate cats: a pulse and a ramp-and-hold stretches. The pulse stretch elicited only a short-latency response from the motoneurones while the ramp-and-hold stretch gave rise to two bursts of reflex EMG (dynamic and static stiffnesses). The reflex-mediated dynamic stiffness results, presented in Fig. 5.6*A*, reflect the decrease seen in the reflex responses to the pulse stretch (Fig. 5.4) when plotted against background force. In

contrast, the reflex-mediated static stiffness presents a nonlinear modulation with background activity level: a slight increase at low forces, a peak in the middle of the range, and a decrease with strong muscle contractions.

These contrasting behaviours in the reflex response suggest that the two types of reflex may play a different role in the regulation of joint stiffness. Following a series of experiments in the cat soleus muscle, Nichols and Houk (1976) proposed that the stiffness may be the regulated variable of the muscle feedback systems and that the main function of the autogenetic reflexes would be to compensate for any variations in the muscle intrinsic properties. Studies of short-latency reflexes with human ankle extensor muscles (Allum & Mauritz, 1984; Stein & Kearney, 1995) and first dorsal interosseous muscle (Carter *et al.* 1990) further supported this hypothesis. Moreover, they confirmed that the short-latency reflex do not only compensate for the variations in intrinsic stiffness due to a change in background muscle activity, but also compensate for the muscle yield observed during the stretch, keeping the stretch resistance at a constant level. Our results also support this hypothesis since the reflex-mediated dynamic stiffness decreased while the contraction level increased (with a concomitant increase in intrinsic stiffness).

In contrast, a linear increase in short-latency reflex response was observed over the entire range of tonic forces studied in the human elbow flexors (Stein *et al.* 1995). This positive relationship between reflex force and contraction level, known as 'automatic gain control' (Matthews, 1986), seems to also happen during locomotion in the cat triceps surae muscles (see Fig. 5.5). The reasons for such discrepancies between muscles remain uncertain although the importance of some of these muscles in postural control could lead to an explanation.

For the reflex-mediated static stiffness, an 'automatic gain control' of the mechanical response was demonstrated to work for a range of background forces up to 50% of a maximal voluntary contraction (MVC) in human ankle dorsiflexors (Sinkjær *et al.* 1988), and extensors (Toft *et al.* 1991) as well as for up to 50% of the range of operating forces in the cat soleus muscle (Hoffer & Andreassen, 1981). Beyond this point the reflex-mediated static stiffness gradually declined towards zero. Our results are comparable to these findings.

Although not clearly shown in the present study, the averaged EMG and static stiffness increments caused by a longer latency reflex were linearly correlated for low operating forces but diverged at higher forces, suggesting a saturation in the contractile apparatus rather than in the segmental reflex pathway (Hoffer & Andreassen, 1981; Toft *et al.* 1989). As an explanation for this discrepancy between EMG response and reflex-mediated stiffness, it was suggested that for high contraction levels, most motor units are firing close to their fusion frequency. Therefore any additional EMG activity in the form of a modulation of their firing rate would be ineffective in generating additional torque (Merton, 1954; Woods, Furbush & Bigland-Ritchie, 1987).

5.4.3 Gain of the stretch reflex loop

The gain of the stretch reflex loop was estimated for the pulse stretches using a dynamic reflex model. As expected, the estimated gains reflected the measurements of the reflex force response for both tonically and phasically active TS muscles (compare Figs. 5.4 and 5.10). During tonic contractions, the loop gain was large at low contraction levels and decreased when the background force increased. In contrast, the loop gain was

substantially lower and rather independent of the muscle activation level during locomotion. This difference in the gain, added to a difference in the phase lag, reinforces the idea that separate control mechanisms are affecting the reflex pathway and altering the reflex contribution to the total muscle activation recorded during postural and locomoting tasks (for more details see DISCUSSION in CHAPTER 4).

An important result about the reflex loop gain appears when comparing the magnitude of the loop gain calculated with the short pulse data (Fig. 5.10) with the gain as measured with the sinusoidal stretch data (see Fig. 4.7 in CHAPTER 4). The gains reported here are much larger than those measured with the sinusoidal stretch for both tonic and phasic contractions. A rapid pulsatile stretch therefore evokes a stronger reflex response than a more continuous perturbation such as the sinusoidal stretch. Stein and Kearney (1995) examined the short-latency reflex in human ankle extensors superimposing random perturbations to pulse displacements. They demonstrated that the amplitude of the reflex response was substantially reduced in the presence of random continuous perturbations.

In the course of analyzing the current data, an attempt was made to use the muscle characteristic parameters evaluated from the sinusoidal stretch data (see CHAPTER 4) in order to simulate the response to the pulse perturbation (with data acquired from a cat for which we experimentally applied both pulse and sinusoidal stretches). The simulated traces, although similar in shape, presented much smaller reflex responses as compared to those obtained experimentally. This difference clearly highlights the nonlinear characteristic of this reflex system, and emphasizes the fact that results obtained with one type of stretch cannot be automatically transposed to predict the reflex response to other types of perturbation.

5.4.4 Effect of external load stiffness

Using a torque motor with an adjustable compliance, the reflex responses to the pulse stretch were examined with different external load stiffnesses. The length and force results that we obtained are comparable to those obtained by Stein *et al.* (1995) when they looked at the short-latency stretch reflex in human elbow flexors. The effect of the external load stiffness on the gain of the reflex loop was mostly evident at rest and during tonic contractions. The magnitude of the loop gain increased as the load became more compliant, especially for low levels of background activity. In contrast, no appreciable differences in the magnitude of the loop gain were discerned between the various load stiffnesses with phasic contractions. Indeed, the reflex gain was kept low during locomotion whatever the stiffness of the external load.

These data further support the hypothesis suggesting the presence of task-dependent control mechanisms of the reflex loop. It appears that, during walking, the involved mechanism (most likely presynaptic inhibition) exerts a great control in keeping the reflex response small. On the other hand, it seems that the mechanical properties of the muscle and load are more influential in the regulation of the reflex size during tonic contractions.

The results shown in Fig. 5.12 were at first surprising, proposing that for external loads of 0.5 and 1.5 mm/N, there should be instability in the system (the gain being greater than 1 with a 180^0 phase lag during tonic contractions). Although small oscillations were sometimes seen during the experiments, they did not happen with every cat, and never with a stiffer (or isometric) loading. The fact that tremor was generally not present could be due to the existence of rectification in the feedback loop. This rectification nonlinearity

was believed to be responsible for preserving stability at the elbow joint, despite an elastic loading (see simulation results in Stein *et al.* 1995).

Much evidence has been presented to support the participation of segmental reflexes in the generation of physiological tremor (for reviews see Rack, 1978; Allum, 1984). Without excluding any other mechanisms, it has been proposed that tremor is most likely due to segmental reflexes either with twitch-generated afferent activity when isometrically contracting (Prochazka & Hulliger, 1983), or with a combination of agonist-antagonist reflex response (Joyce, Rack & Ross, 1974). However, it appears that the instability of the feedback loop occurs and even dominates under a variety of physiological loading conditions. When a spring-mass system was used as the external load, the tremor frequency was strongly related to the mechanical properties of the limb-load system (in humans: Joyce & Rack, 1974) or muscle-load system (in decerebrate cats: Nichols, Stein & Bawa, 1978). The results from the present study confirm the fact that the stretch reflex gain is directly influenced by the mechanical characteristics of the external load. However, muscle properties and nonlinearities in the reflex loop help to maintain a certain level of stability. Moreover, segmental control mechanisms (e.g. presynaptic inhibition) seem to play an important role in keeping the stretch reflex gain low during locomotion, preventing instabilities which may occur in the presence of the varying loading conditions experienced throughout the step cycle.

Post Scriptum

The manuscript of this chapter is currently in preparation for publication:

DE SERRES, S.J., BENNETT, D.J. & STEIN, R.B.
Stretch reflex in the triceps surae muscles of decerebrate and spinal cats with isometric and compliant external loading.

I was involved in this project from the beginning and worked on every steps of its realization: experimental setup preparation (including cat surgery), data acquisition and analysis (including writing computer programs to process the data, see APPENDIX B, plus performing the computer simulations), and complete writing of the manuscript.

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CHAPTER 6

GENERAL DISCUSSION

The aim of this study was to address three important questions related to the modulation of reflexes during a rhythmic movement such as locomotion: (1) which neural mechanism is responsible for the phase-dependent reflex reversal observed in the human tibialis anterior muscle during walking? (2) how is the spindle sensitivity of the cat soleus muscle regulated during postural and locomotor activities? and (3) how do the stretch reflex and its gain in the cat hindlimb extensors vary with the type of perturbation, the muscle loading characteristics, and the behavioural task?

6.1 NEURAL MECHANISM FOR REFLEX REVERSAL

A reversal in the sign of a cutaneous reflex during walking was studied in the human tibialis anterior (TA) muscle by recording from single units. Three hypotheses were put forward: (1) functional task groups, (2) reflex groups, and (3) parallel pathways. The first hypothesis relied on the fact that groups of motoneurones innervating some cat muscles were found to be task-specific (Hoffer, Loeb, Sugano, Marks, O'Donovan & Pratt, 1987; Chanaud, Pratt & Loeb, 1991; Gordon, Loeb & Richmond, 1991). The second hypothesis suggested that the motor units would be grouped, not on the basis of

their functional recruitment but rather dependent on their cutaneous reflex pathway. Finally, the third hypothesis did not differentiate among groups of motor units, and only supposed that a balance in the strength of parallel interneuronal pathways was responsible for the reflex reversal (Eccles & Lundberg, 1959; Schomburg & Steffens, 1986).

The results strongly favour the third hypothesis since (1) the majority of the units recorded from during undisturbed walking were active during both phases of the step cycle (leaving out the first hypothesis), and (2) post-stimulus time histograms indicated that the same motor unit was excited during the swing phase and inhibited during the transition from swing to stance (dismissing the second hypothesis).

6.2 REGULATION OF SPINDLE SENSITIVITY

Fusimotor control was indirectly studied in reduced preparations by recording from soleus muscle spindle afferents in premammillary decerebrate cats during tonic contractions and, after spinalization, during locomotion produced by either Clonidine or DOPA. At rest, in all preparations, the firing rate and modulation in primary muscle spindle afferents were generally much higher than after deefferentation, suggesting a predominant dynamic γ-drive. During tonic contractions, in decerebrate cats, primary afferents generally fired with much higher mean rates and lower sensitivities, suggesting a large increase in static γ-drive (possibly accompanied by a small decrease in dynamic γ-drive). When locomotion commenced, in spinal cats, a substantial increase in primary

afferent firing rate along with a decrease in sensitivity again suggesting the presence of an increased static γ-drive.

Even though the inferred increase in static γ-drive occurred at the onset of locomotion for both Clonidine- and DOPA-treated spinal cats, some differences were seen in the pattern of activation of this γ-input: tonic activation for Clonidine-treated cats, and phasic activation (more or less in phase with the α-activity) for the DOPA-treated cats. Moreover, unlike DOPA, Clonidine decreased and even eliminated the presumed resting dynamic γ-drive in many afferents for both decerebrate and spinal states. This effect on γ-drive may account, at least in part, for the suppressive effect of Clonidine on spasticity in humans (Nance, Shears & Nance, 1985; Weingarden & Belen, 1992).

Although there were some common features, such as predominant static γ-drive during both tonic and phasic contractions, the pattern of static and dynamic γ-drive was not closely linked to the α-activity under the conditions studied: γ-drive without α-activity (during tonic contractions in untreated decerebrate cats), and α-activity without a significant γ-drive (also during tonic contractions, but in Clonidine-treated decerebrate cats, shown for the first time). These results support the concept of 'fusimotor set' (Prochazka, 1989), showing flexibility in the γ control for specific task requirements.

6.3 MODULATION OF STRETCH REFLEX

The triceps surae (TS) stretch reflex was studied in decerebrate cats during crossed extensor stimulation and, after spinalization, during rhythmic locomotor activity induced by Clonidine. Three types of perturbation were applied to the TS muscles: a sinusoidal stretch, a brief pulse, and a ramp-like perturbation. Using both the sinusoidal stretch and the pulse input, the phase-dependent modulation of the Ia monosynaptic reflex, seen during walking in humans (from H-reflex studies: Capaday & Stein, 1986; Crenna & Frigo, 1987; Yang & Whelan, 1993), and in decerebrate cats (for both stretch and H reflexes: Akazawa, Aldridge, Steeves & Stein, 1982), was still present after spinalization. These results strongly suggest that the neural mechanisms involved in this modulation have a segmental origin.

During locomotion in spinal cats, afferent feedback from low frequency ankle movements reflexly produced a small but significant fraction of the extensor force (23% of the stretch related force modulation; see also Yang, Stein & James, 1991). This fraction was remarkably constant at the different contraction levels of the step cycle. In contrast, during tonic contractions, afferent feedback produced up to 50% of the total muscle force during perturbations, clearly contributing to the maintenance of posture.

Both dynamic (from primary afferents) and static (from both primary and secondary afferents) stiffnesses were measured at different levels of tonic contraction in decerebrate cats. The reflex-mediated dynamic stiffness gradually decreased with increasing background force while the reflex-mediated static stiffness displayed a nonlinear behaviour with a slight increase at low forces, a peak in the middle of the range, and a

decrease with strong muscle contractions. These contrasting behaviours in the reflex response suggested different roles in the regulation of joint stiffness for these two reflexes.

The gain of the stretch reflex loop was estimated with both sinusoidal stretch (using the experimental data) and pulse input (via computer simulations). The loop gain reflected the differences in the reflex responses observed between tonic and phasic contractions. However, the gains obtained from the pulse stretch data were much larger than those evaluated with the sinusoidal stretch, highlighting a nonlinear characteristic of this reflex system (Stein & Kearney, 1995). Finally, the loop gain was strongly affected by the mechanical characteristics of the external load during tonic contractions. The more compliant was the load, the higher was the gain. However, this dependence was greatly attenuated during locomotion, suggesting that other neural mechanisms (e.g. presynaptic inhibition) are acting in order to keep the stretch reflex gain low, in spite of the variable loading conditions occurring throughout the step cycle.

6.4 CONCLUSIONS

The studies presented in this thesis have once more shown evidence for the complexity of the mechanisms involved in modulating the reflexes during postural and locomotor activities. The results highlighted the various sites in the reflex loop where the modulation might occur. The stretch reflex studies indicated a dramatic difference in the reflex behaviour when comparing postural and locomotor tasks. These results suggest that presynaptic mechanisms are certainly involved in attenuating the reflex during locomotion,

whereas a variation in the motoneuronal excitability is likely to play a role in the modulation observed within the step cycle. Fusimotor control is also important, demonstrating flexibility between the tasks with a predominant dynamic γ-drive prior to movement, a considerable increase in static γ input at the initiation of the movement, and an indication of a phasic fluctuation in dynamic γ-motoneurone activity during walking (observed in the DOPA-treated spinal cats). A more thorough study, closely comparing the stretch and H reflexes, will certainly provide further information regarding the implication of the gamma system in the control of the reflex responsiveness during these movements.

The study on the phase-dependent reversal of a cutaneous reflex provided evidence that such a reflex modulation is controlled at the interneuronal level. Even though there are no interneurones interposed between the primary afferents and the motoneurones in the monosynaptic pathway of the stretch reflex, the role of interneurones in the task- and phase-dependent modulations seen in the reflex might be substantial. Indeed, interneurones are involved in events occurring presynaptically (primary afferent depolarization [PAD], e.g. see Gossard & Rossignol, 1990), postsynaptically (Renshaw and Ia reciprocal inhibition, e.g. see Pratt & Jordan, 1987), and in regulating the γ-motor system (from muscle, skin and joint receptor afferents, e.g. see Johansson, 1985). These multiple implications of interneuronal relays emphasize the powerful, albeit flexible, motor control happening in spinal neuronal systems.

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APPENDIX A

MODEL OF THE STRETCH REFLEX LOOP

To estimate the gain of the reflex feedback loop in each experimental condition in CHAPTER 5, (external load stiffness, cat preparations and level of background muscle activity), we used a reflex model (modified from A. Prochazka, D. Gillard & D.J. Bennett, unpublished work) as illustrated in Fig. A.1. In the first section of this APPENDIX, a description of the model will be presented, including the details of how parameters were chosen and varied throughout the simulation procedures. The second section contains the details of the gain calculation with both isometric and elastic external loading.

A.1 STRETCH REFLEX MODEL

Elements included in the model are described below, with an indication of how each parameter was selected (Fig. A.1).

[1] Primary afferent (Ia) feedback includes the following transfer function obtained from Matthews and Stein (1969):

$$0.1 \cdot (s+10)$$

This block also includes an adjustable gain and a reflex delay accounting for conduction time from the spindle to the spinal cord, synaptic transmission, conduction time back from the spinal cord to the muscle (via the motoneurones) and excitation-contraction coupling duration. The delay was set at 17 ms for all the experimental conditions while the gain was adjusted for each simulation. Note that the spindle transduction of displacement was for the muscle fibres only: length changes of the tendon were subtracted from the total muscle length.

[2] A rectifier allows only the positive content of the spindle signal to pass through simulating the fact that spindle receptors respond to muscle lengthening only (not shortening). Also, physiologically, they never fire with negative frequencies.

[3] A baseline offset element was introduced to reflect the initial length dependence of muscle spindle receptors. A positive baseline value offsets the Ia signal allowing more of its content to pass through the rectifier, mimicking an initially stretched muscle. This baseline offset, with values ranging from -2 to +2, was set for each experimental state of the cat (decerebrate or spinal), since the twitch test used to optimize the muscle length was performed at the beginning of the experiment and after spinalization. Note that this baseline offset is subtracted after the signal has passed through the rectifier.

[4] Muscle force produced by the motoneuronal activation in response to Ia afferent excitation is imitated by a first-order active component which includes a rate constant representing the exponential force decay. This rate constant was varied throughout the simulations to optimize the goodness of the fit.

[5] A Hill-type parallel stiffness component (Hill, 1938) comprises stiffness and viscosity parameters and is represented by the following equation:

$$B_p \cdot (s + k_p/B_p) \cdot C_{bf}$$

where B_p is the parallel viscosity, k_p is the parallel stiffness, and C_{bf} is a background force factor. B_p and k_p were initially taken from Bawa, Mannard and Stein (1976) for plantaris muscle, and were adjusted in the present study for simulating the three muscles stretched (TS). Once determined for each cat, B_p and k_p were kept constant for all the simulated trials. Note that K_p/B_p constitutes the viscoelastic rate constant for the loop. C_{bf} was introduced to account for the background force dependence of the parallel stiffness and was adjusted for each simulated trial (ranging from 0.8 to 5.1).

[6] Another discrete element models the series tendon compliance, $1/k_s$, where k_s is the series stiffness. As k_p, k_s was set for each cat, based on the data from Bawa *et al.* (1976b), and was amplified by C_{bf} to account for the change in stiffness with change in background muscle force.

[7] The external load was either very stiff (isometric) or compliant, and its characteristics were expressed by the following transfer function:

$$\frac{1}{B_e \cdot s + k_e}$$

where B_e is a damping factor (mimicking the damping of the torque motor) and k_e is the external load stiffness. B_e was selected with a trial-and-error method

attenuating undesirable oscillations, while k_e was experimentally determined using the following equation:

$$\frac{1}{k_e} = \frac{L_{nominal} - L_{int}}{F_{int}}$$

where $L_{nominal}$ (in mm) corresponds to the nominal amplitude of the stretch from the motor controller, L_{int} (in mm) corresponds to the actual stretch amplitude as measured for each trial during the experiment, and F_{int} (in N) corresponds to the intrinsic force produced by the parallel and series elastic elements of the muscle-tendon complex during the stretch. As mentioned earlier (see METHODS) the nominal stretch amplitude was varied according to the load compliance, in order to keep stretching the muscle by approximately 1 mm. The external load stiffness was calculated at different levels of background muscle activity, and the results from this calculation, presented in Fig. A.2 for one cat, showed that k_e slightly increased with increasing background contraction. In Fig. A.2, stiffness values corresponding to the 0.2, 0.5 and 1.5 mm/N compliances are indicated by dashed lines.

[8] The external perturbation (muscle stretch) consisted in a length stretch of variable nominal amplitude (adjusted such that the actual muscle stretch was always 1 mm even with elastic loads), transformed into a force perturbation.

Total muscle force and length, computed during the simulations, were used for comparing the experimental and simulated data traces. The simulation parameters were set for a 450 ms run time of the Euler algorithm with minimum and maximum steps of 1 ms. Terms were added to the transfer functions for the Ia receptors [1] and the parallel stiffness element [5] in order to have at least the same number of poles than zeroes, a MATLAB requirement for running the simulations.

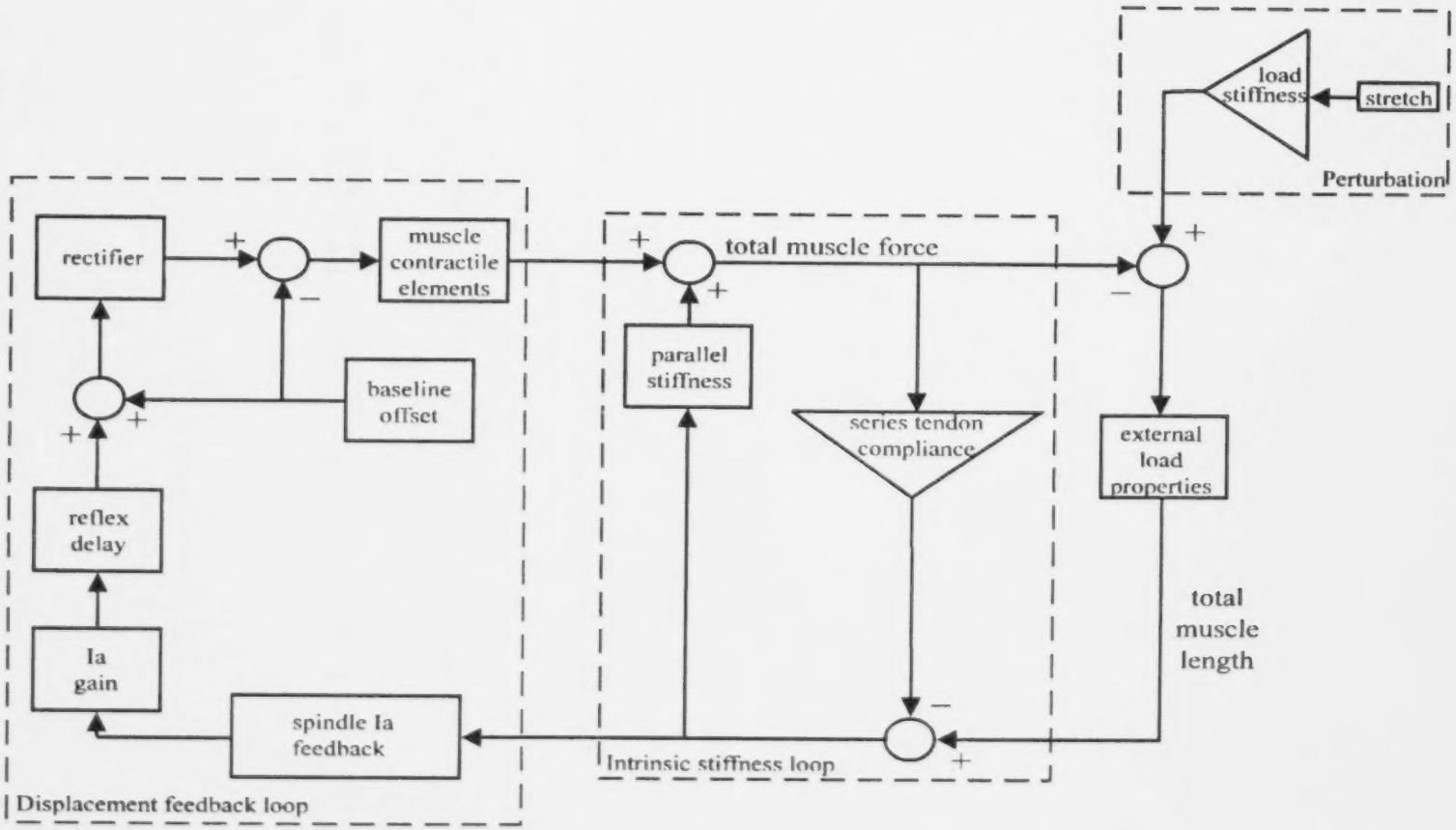

Figure A.1 - Schematic diagram of the reflex loop model.
Schematic representation of the reflex loop model used to simulate the experimental data obtained in response to a pulse stretch.

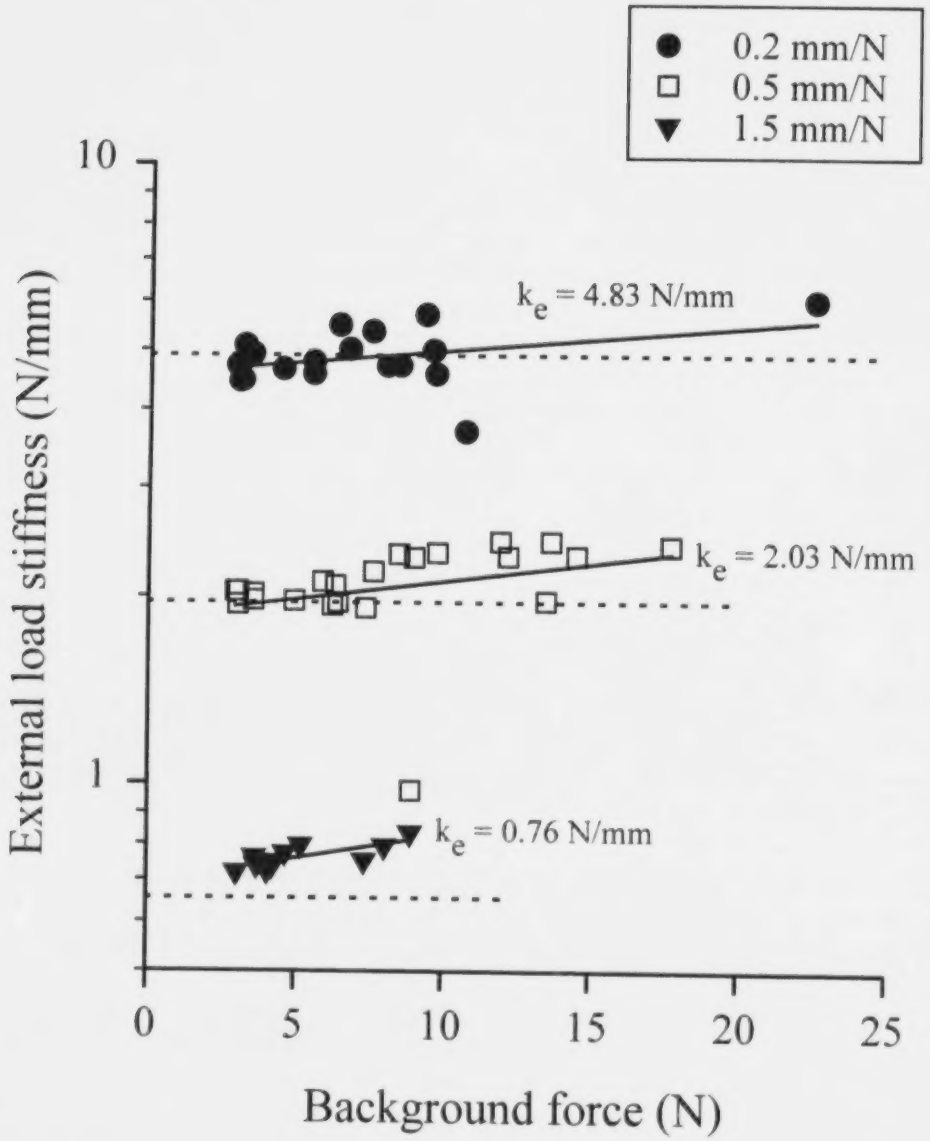

Figure A.2 - External load stiffness as measured experimentally.
External load stiffness measured experimentally with pulse stretch, plotted against background force. Data from one cat.

A.2 REFLEX LOOP GAIN CALCULATION

The gain of the stretch reflex loop was evaluated for each experimental condition in which parameters were determined to closely fit the experimental data. The procedure used to measure the reflex gain consisted of two steps and followed the same principles than those behind the gain calculation from experimental data obtained by sinusoidally stretching the muscles (see Chapter 4), or stretch reflex gain estimation in the human elbow joint (Bennett, Gorassini & Prochazka, 1994).

In the first step, a sinusoidal input was introduced to simulate a stretch of the whole muscle. As mentioned above, the length change taken by the tendon was subtracted from this total length change prior to be transduced by the spindle. The force produced by the activation of the spindle receptor (stretch) was measured (top of Fig. A.3). For the second step, this force was fed through the mechanics of the system, including intrinsic muscle properties (parallel and series stiffnesses) and external load characteristics (thick arrow from top part to bottom part of Fig. A.3). The output consisted in the total muscle length computed at the exit of the external load transfer function. Note that for the isometric loading condition, the output was computed as the length change in the tendon since the total muscle length was invariant because of the very rigid load.

Due to the rectification element present in the model, the output was usually not a perfect sinusoid. Indeed, at low frequencies, almost half of the sinusoid was truncated. The output signal was therefore optimally fitted with a perfect sinusoid (by linear least squares method; Norton 1986).

The magnitude (M) and phase (θ) of the loop gain were thus obtained for each simulated trial as follows:

$$M = \frac{A_{output}}{A_{input}}$$

$$\theta = \theta_{output} - \theta_{input}$$

where A_{input} and θ_{input} were the amplitude and phase, respectively, of the sinusoidal input fed to the model, and A_{output} and θ_{output} were the amplitude and phase, respectively, of the sinusoid best-fitted to the model output. This open loop calculation could not be performed in only one step since opening the intact reflex loop shown in Fig. A.1 also opens the intrinsic loop of the muscle elastic elements, loop which is always intact in the biological system.

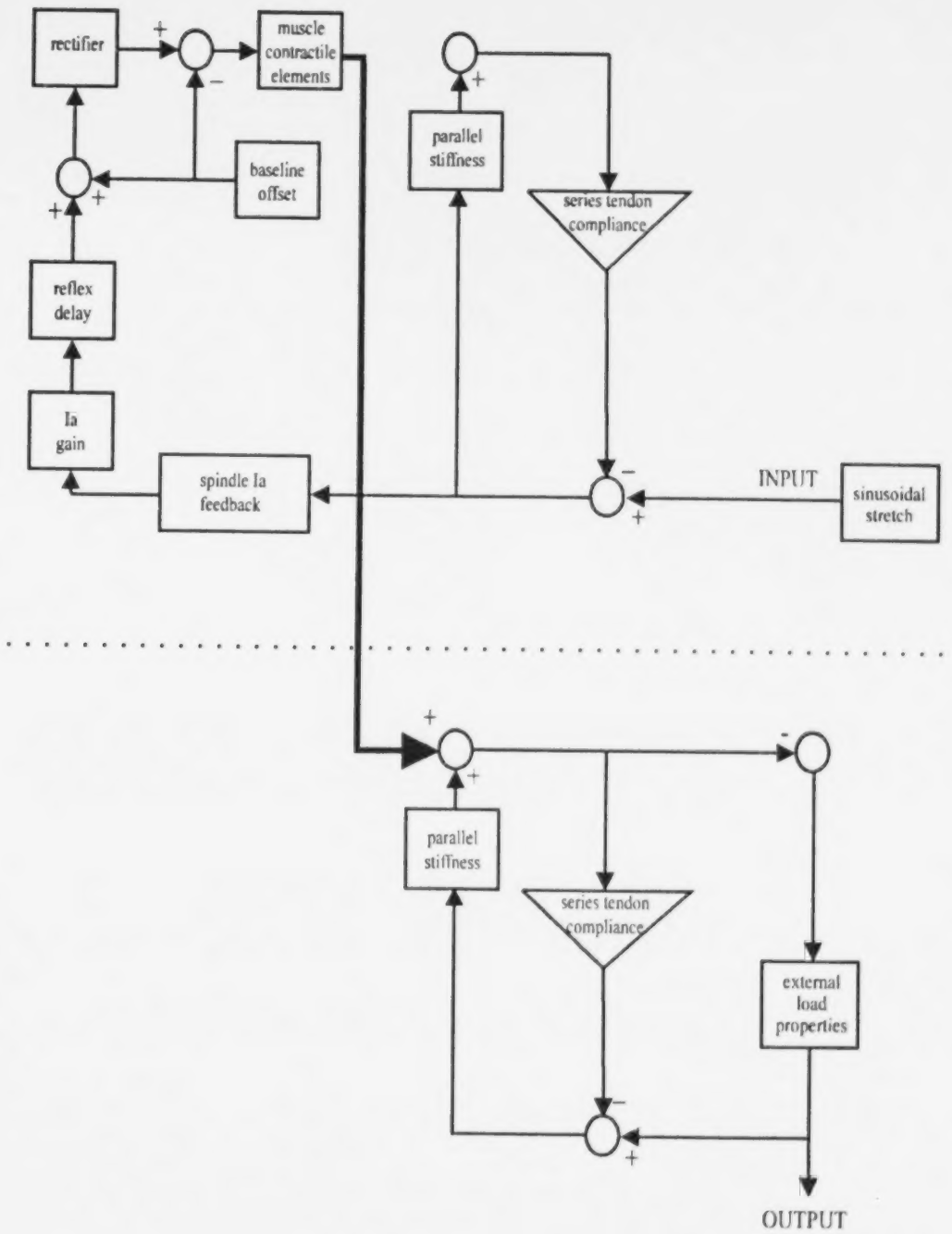

Figure A.3 - Schematic diagram of the modified reflex loop model for gain calculation.
Schematic representation of the reflex loop model, after modifications to estimate the reflex gain.

A.3 REFERENCES

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APPENDIX B

USER'S MANUAL FOR PROGRAM "SOPHIE"

B.1 INTRODUCTION

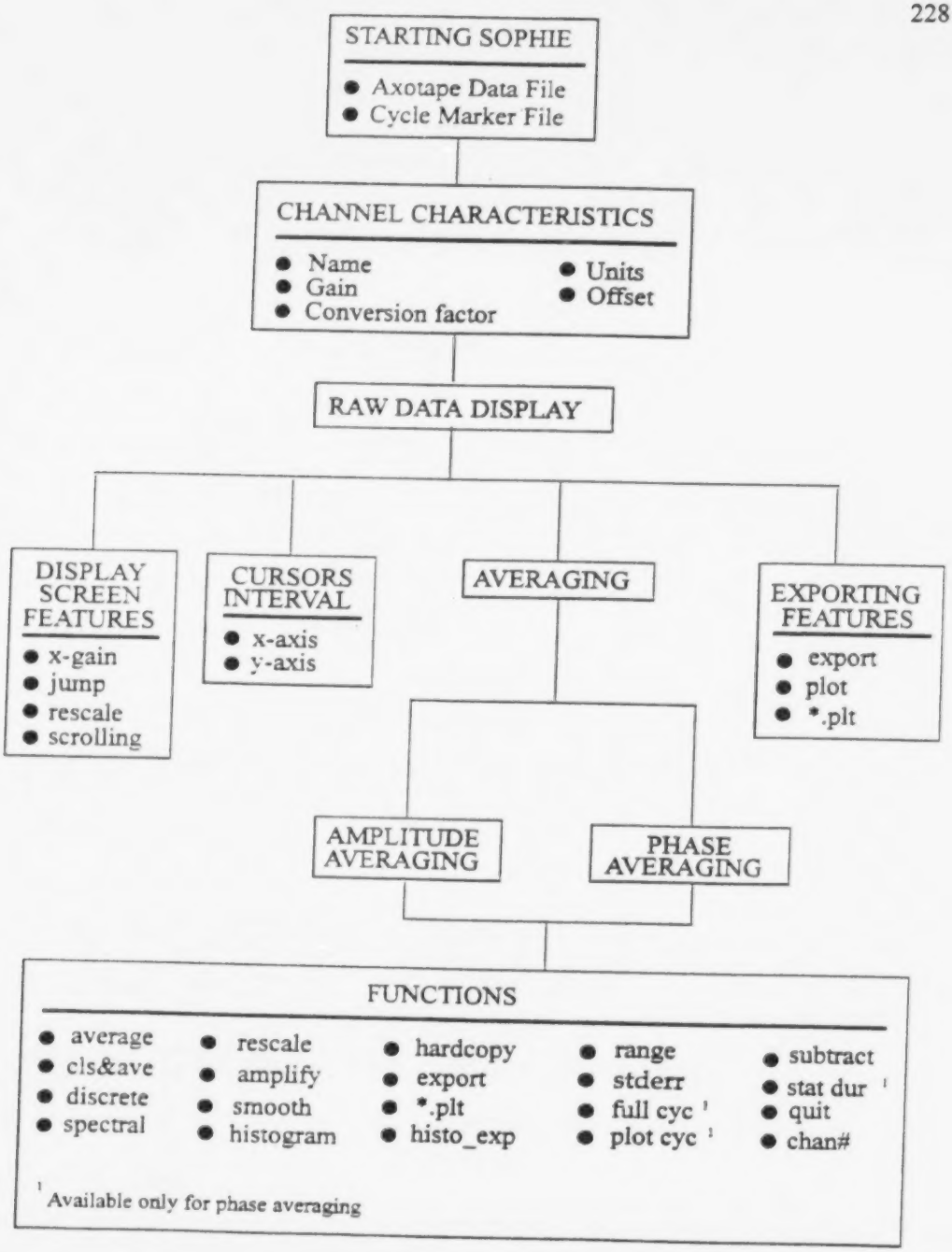
B.1.1 An overview

This program has been written for displaying and averaging data recorded with the software AXOTAPE (Axon Instruments, Inc., Foster City, CA, USA). Averages can be performed based on either a level of amplitude in one recorded signal (Amplitude average) or the time of occurrence of an event within a step cycle (Phase average). This program can handle up to 8 channels simultaneously. In the case that more than 8 channels have been recorded on one AXOTAPE file, there is a possibility of generating two files from the first one, each file having less than 8 channels. This procedure is performed with the AXOTAPE software. IMPORTANT: The two files obtained this way have to contain the same number of channels. Note that the channel numbers on the Axotape interface connector board range from 0 to 15. The program will work as long as there is no more than 8 channels recorded from; the channel numbers may still range from 0 to 15. For example, one can record from channels 1, 4, 5, 8, 12, 13 and 15, a total of 7 channels.

If this program is used for phase averaging, it is a prerequisite that the user run the program SELPOS prior to SOPHIE. With the user's help, SELPOS will create a file which will contain the positions of markers that define the start and end of each individual walking cycle.

With AXOTAPE, the data is stored in numerical files in A/D units (analog to digital conversion units). At the time of reading the data file, the program SOPHIE transforms this data into millivolts (mV) for every channels. Remember that AXOTAPE requires full scale input to be ±10 volts.

This program runs well on a 386 or 486 IBM-PC computer. It requires a 2 or 3 button mouse to work effectively. Every output to a printing device will work correctly with a Hewlet Parkard LaserJet III printer. Other printers may work but the program has not been tested on them yet. This program reads data files from AXOTAPE generated by two different versions of the software: Version 1.2, 16 channel TL-125 model (older version) and version 2.0 (newer version). The two major differences between these two versions are the A/D converter resolution, and the order of labelling the channel numbers.



B.1. Schematic list of the program functions.

B.1.2 Program structure

A schematic list of the program functions is displayed on Fig. B.1. The program's order of execution is illustrated from top to bottom. Some functions are available to display, select, and export portions of raw data. Other functions are reserved for the averaged data, after performing an amplitude or phase averaging.

B.2 STARTING "SOPHIE"

Start the program by typing SOPHIE at the DOS prompt (the executable file is called SOPHIE.EXE). Type in a name of a file containing data which was digitized with AXOTAPE. Information concerning the data file is displayed on the screen. This information is read in the header portion of the raw data file and corresponds to the parameters used at the time of recording. If the program is used for phase averaging, type the name of the file which contains the marker positions (created with SELPOS).

B.2.1 AXOTAPE data file

Data filename (raw data file, w/o ext): Answer to this question by typing the name (without the extension) of the data file as recorded by AXOTAPE. If the data file is in another directory than the current one, the user is required to indicate the path.

Comment: Comment which was typed be the experimenter at the time of saving the AXOTAPE data file.

Number of channels: Total number of channels at recording (no more than 8 channels is required to use this program).

Clock period (μsec): The clock period is the sample time in microseconds. This is the clock period used by the AXOTAPE A/D converted for sampling all the channels. It has to be multiplied by the total number of channels to obtain the sampling interval for each channel (trace).

Sampling frequency (Hz): This number represents the sampling used during the recording of the data. This sampling frequency is the same for all the channels.

Elapsed time (sec): Total length of time of data recording.

Number of tags: Number of tags that were recorded simultaneously with the data digitization (AXOTAPE).

B.2.2 Cycle marker file

Do you want to retrieve a step marker file [y/n]? Answer 'y' for phase averaging. A step marker file is necessary for phase averaging.

if 'y' was answered to the previous question

Name of step marker file (w/o extension): Enter the name of the marker file that was generated by SELPOS.

B.3 CHANNEL CHARACTERISTICS

The next window display the characteristics proper to each of the channels recorded by AXOTAPE. The user is offered the opportunity to save a current characteristic settings for further use. Channel characteristics that are identical and applicable to a series of files can be saved in one file which can then be retrieved when needed. In a similar way, the user can retrieve a characteristic file which contains some specific characteristics that have been previously saved.

In order to change any of the default settings, type a green letter. You will then be asked to type the number of the channel for which you want to change a characteristic. Similarly, if typing 's' or 'r', the user will be asked to type the name of the file to retrieve or save the channel characteristics.

Characteristics such as gain, offset and conversion factor will calibrate all y-axis scale bars in the subroutines appropriately. These characteristics include:

CHANNEL #: The number of the channel (labelled from 0 to 15).

NAME: The name of the channel as entered by the user in the configuration file at the time of data digitization (AXOTAPE). It can also be edited by the user.

ORIGINAL GAIN: Gain of the channel which should combine both the gain at recording (if the data was initially recorded on a video tape) and the gain at digitization. The program will take this gain into account to display the real value of the data. The default gain is 1.

OFFSET VALUE: Offset of the channel trace. This number will allow the user to remove an offset included in the data at recording and/or digitization. The program will take this offset into account to display and average the data. The offset is a value that is subtracted from the recorded data. The default offset is 0.

NOTE: The offset has to be expressed in the units selected for the corresponding channel and as it should be after transformation with gain and conversion factor. The ideal way of determining this offset is to not input any at first, make all the changes of the other characteristic numbers for a specific channel, save them and move to the next screen. Then, using the mouse and rescaling functions (see section B.4 Display of Raw Data), the user can estimate the offset, quit the program and get back in with the same data file. Then, he (or she) can input the offset after retrieving the characteristic file previously saved. Don't forget to same your characteristic file again!

CONVERSION FACTOR: A conversion factor can be typed here to transform the data from mV to any other units. For example, it is possible to transform a length trace from mV to mm by typing the appropriate conversion factor. This factor corresponds to the number of mV per selected unit (for example, number of mV in 1 mm). The default conversion factor is 1.

UNIT: The type of units for which the data has been transformed by the conversion factor entered in the previous characteristic category. For example, the units for a length signal could be 'mm'. The type of units chosen should match the conversion factor indicated for each specific channel. The default units are mV for every channels. Note that the use of Greek letters such as μ in μm for micrometer, is not recommended.

Typing 'q' brings the user to the next screen which displays raw traces for the current data file.

B.4 DISPLAY OF RAW DATA

The main display screen show traces for all channels. Starting with channel 0 at the top. Due to the descending order of labelling of the channel number in the older version of AXOTAPE (version 1.2), the display number of these channels has been reversed. For example, channel 15 on the A/D converter (AXOTAPE board) became channel 0, channel 14 became channel 1, etc.

The y-axis scale bars are located to the left of each trace. The full scale voltage (±10 V) has been modified by the gain, conversion factor and offset for each channel so that the y-axis bars are in the user's selected units. Each trace has a magenta zero line. The channel number is attached to that zero line and moves with it. At the bottom of the screen are the two boxes at the left and right limits of the traces. The numbers in the boxes indicate where in the file the screen starts and ends (in seconds). Also at the bottom of the screen is an x-axis scale bar representing the scale (ms).

B.4.1 Crossed hair cursors

Mouse cursor

In the top left of the screen is a real time readout of the mouse cursor value (small arrow) for any given channel. As the cursor moves around the screen the values for each channel change appropriately. In this way the y-axis value for any displayed point can be found simply by placing the point of the arrow over the data point and reading out the number from the display in the top left corner.

Included in the upper left display is a value calculated from the difference in the crossed hairs for selected cursor readings. To anchor the crossed hair to any point on the screen you must point to the appropriate point and press the left button on the mouse. To anchor the second crossed hair you must move the cursor to a different point and press the left button again. At this point an 'x' and 'y' difference (YDIF and XDIF) will be calculated for all channels relative to the area demarcated by the crossed hairs.

At this point you can erase the cursors by pressing the middle button on the mouse or you can store the cursor values in the output file selected during the setup by pressing the right button on the mouse. When the values have been stored, a readout of the file location values, the x difference and the word STORED will appear in the lower right corner of the screen. The cursor marks will remain on the screen until a new screen is displayed. At this point new cursor values can be selected, displayed, erased or stored. This procedure may be used instead of running SELPOS, although I strongly recommend the use of SELPOS for marking the rhythmic cycles.

On the right portion of the screen, a list of options appears, options which can be selected either with the mouse or by using the highlighted green letter for each selection. A description of each of these options can be found below.

B.4.2 Display screen features

Scrolling through the data: The user can move through the file to display successive windows in one of two ways. First, moving the cursor off the screen will shift the display ahead and back depending upon if the movement is to the right or the left, respectively. A shift of half a screen (x-axis) ahead or back will be obtained by moving the cursor off the screen towards the right or left TOP HALF of the screen, respectively. A shift of a full screen (x-axis) ahead or back will be obtained by moving the cursor off the current screen towards the right or left BOTTOM HALF of the screen, respectively.

Arrows and ctrl+arrows: Using the left and right arrow keys will shift the screen by 1/8 of a full screen ahead and back, respectively. Using ctrl+arrow will shift the screen one full screen in the same directions as above.

NOTE: It is also possible to jump to a new location within the data file. The JUMP function is described below.

X-GAIN

The display gain can be manipulated in the x direction. The default window contains a trace for each channel and each trace contains 512 points. Therefore, the buffer of data read in the AXOTAPE file has 512*NUMBER OF CHANNELS points. The time length of data is therefore dependent on the sampling rate chosen at the time of digitization. If a larger window is required, the user can select the X-GAIN function. In the lower right corner a prompt is displayed showing the old x-gain and asking for a new one. The value entered by the user has to be an integer ranging between 1 and MAX where MAX corresponds to how many times 512*NUMBER OF CHANNEL points are included in the total amount of points in the data file. An example below show how to calculate this MAX number.

NOTES: 1) When the X-GAIN is 1, every point for each trace is displayed on the screen. If the X-GAIN is 2, every second point for each trace will be displayed on the screen, and so on. This function therefore acts as a display filter.

2) The user should remember that increasing the value of the X-GAIN will NOT affect in any case the data for all the averaging manipulation which will follow.

RESCALE

The display amplitude can be adjusted with the rescale function. After selecting RESCALE the program will prompt (again in the bottom right) for the channel that you would like to rescale. After selecting the channel, the program will prompt for an upper and lower limit. This corresponds to the upper and lower limit (in the selected unit of the channel, as indicated by the used in the characteristics) which you would like displayed in the window provided for that channel.

JUMP

If one needs to move the cursor over a long distance through the data file, the JUMP function is used. JUMP will allow the user to jump to either the position of a tag or to a point corresponding to a time point (in seconds) into the file. These questions will appear in the bottom right corner of the screen for the user to indicate his choice. The amount of seconds entered is an absolute value in the file. For example, if the file contains data for 90 seconds, the user can request to jump to 40 seconds into the file, and then back to 5 seconds, etc.

B.4.3 Exporting features

PLOT

A hardcopy output can be obtained by selecting PLOT in the right panel menu. The PLOT function plots the screen to a LaserJet III printer (or any HPGL compatible printer). The traces are printed as they appeared on the screen, for the same section of data.

EXPORT

An ASCII output of all traces can be obtained by selecting EXPORT from the menu. EXPORT will prompt for a file name in which to store the ASCII data. The extension **.EXT** will automatically be added to the filename given by the user. The program stores the data in columns; the number of columns corresponds to the total number of channels plus one. The first column contains the data point of the time vector. The other columns contain the data, one column per channel, starting with the

lowest channel number. Every sampled point will be sent to the ASCII file, even if on the screen, only every 5 points are displayed due to an X-GAIN of 5.

Remember, the ASCII file will be stored in the current directory if you do not indicate a new path.

***.PLOT**

This function allows the user to save the content of the screen in a file compatible with the software CORELDRAW (graphic drawing file). The user will be prompted to input a name for the file, without the extension. The extension **.PLT** will be automatically added to the filename provided by the user. The program also provides flexibility for the user regarding the sampling interval with this function. This means that a different sampling rate can be chosen for each channel. For example, if a data file contains 3 EMG signals, a force and a length signals, it was most likely digitized with a sampling frequency of a minimum of 500 or 1000 Hz, due to the electromyographical signals. This high sampling rate was probably not necessary for the length and force signals. Therefore, the user can here select a different sampling rate for each individual signals, saving more points for an EMG signal as opposed to the length and force signals. The user is prompted to select this sampling rate for each individual channel. A selected sampling rate of 5 will mean that every 5 points will be saved in the graphic file. The maximal value that can be selected will be equal to the value of X-GAIN.

B.4.4 Averaging the data

AVERAGE or AMPLIAVE

This function averages the data grouping it upon the amplitude values of one specific channel, as selected by the user. Full details concerning the use of this function are given in the next section (B.5 - Normal averaging function).

PHASEAVE

This function averages the data grouping it upon the time of occurrence of an event in a cycle of a rhythmic movement. Please note that this function can only be used after a file containing the markers indicating the positions of these cycles has been generated with the program SELPOS. Also, the user has to have answer 'y' to the question "Do you want to retrieve a step marker file [y/n]?" and to have supplied the name of that file, at the start of the program. Full details concerning the use of this function are given below (B.6 - Phase averaging function).

QUIT

This function allows the user to quit the program and return to DOS.

B.5 AMPLITUDE AVERAGING

B.5.1 An overview

This function averages the data based upon the amplitude of a signal (from one of the recorded channels) at the time of occurrence of an event. For example, it is possible to average reflex responses by grouping them according to the level of background force or EMG activity in the muscle that was stimulated, at the time of stimulation. A set of parameters can be selected by the user, providing substantial flexibility in the averaging procedure. Default parameters are already selected. Details about these parameters are given below.

B.5.2 Parameters

The following is a list of all the parameters which have to been selected by the user in order to average the data based on the amplitude of one signal at the time of occurrence of the stimulus. Some of these parameters need to be determined only if a previous parameter was appropriately selected. In the case that a parameter is not required, it will not appear on the screen. All parameters have been attributed a default value. The user can change any of these values. The user can also save a list of parameters that are specific to a group of data files. These saved parameters can be retrieved when reopening the same data file, or opening new data for which a similar averaging will be performed.

A modification can be done by typing the letter (green) preceding each parameter line. In typing 's', the user can save the current set of parameters for a later use. The same way, in typing 'r', the user can retrieve a parameter file which had been previously saved. Each of these requests have to be followed by <enter>. When satisfied with the parameter selections, the user can type 'q' to quit.

Note that these parameter values are kept as determined by the user as long as they are not changed, or that the user does not quit the program (return to DOS).

Interval information

A. Number of intervals [1-16]:

Number of bins in which the spike-triggered samples will be distributed.

B. Channel for interval distribution:

Channel number for the signal which will be used to distribute the spike-triggered samples. Only one channel can be used for binning the data.

C. Delay before stimulus artefact: ms

The distribution of the spike-triggered samples will be based on an averaged value of the signal preceding the stimulus. The user is required to indicate at what point in time (how many milliseconds prior to the stimulus artefact) this average has to start.

D. Length of time used to determine the interval: ms

How many milliseconds are necessary from the time indicated in (C) to evaluate the mean value of that signal just before the application of a stimulus. It is suggested to allow for more time for an EMG mean than for a force or a length signal.

E. New limits [y/n]:

By answering yes to this question, the program will automatically evaluate the limits of each of the intervals based on the maximal and minimal mean values calculated in the time period indicated by points (C) and (D). These limits will be displayed on the screen after being calculated. The user can then take note of the maximal and minimal values for later analysis. By answering no, the user will be requested to indicate the maximal and minimal value of the limits. The program will divide the range given by these two values in a number of bins of equal size.

F. Minimal limit: Maximal limit: .

These limits will be used to determine the range of amplitude covered for each interval (bin) for a proper distribution of the spike-triggered samples. The user has to indicate the maximal and minimal limits if 'no' was answered at the preceding point.

Stimulus information

G. Stimulus channel:

Input the number of the channel which will be used as the stimulus channel. The program will search on the signal recorded in this channel for a threshold crossing (fixed at the next point).

H. Stimulus threshold: (unit)

The user has to indicate what is the threshold (expressed in the units used by the channel selected in the previous point) at which the program will detect a stimulus. The program will mark the position of a trigger when the signal recorded in the stimulus

channel crosses above the threshold value. No other trigger position will be marked until the signal goes below the threshold value. If the user would prefer marking a trigger position when the signal crosses below the threshold value rather than above, it is possible, but indirectly, by inverting the stimulus signal indicating a negative gain value in the characteristic parameter for this specific channel. In any case, a threshold value can be evaluated by the user in the Raw Data Display Screen, using the mouse cursor to find the appropriate threshold.

I. Distance of real trigger after artefact: ms

In the case when the stimulus artefact used to trigger the spike-triggered averaging came much earlier than the actual electrical of mechanical perturbation, it may be appropriate to move the position of this artefact forward to get closer to the real time of application of the stimulus. This will affect the position of the period of time used to estimate an amplitude level for the purpose of distributing the spike-triggered samples in different intervals. This increase time difference between the stimulus artefact and the application of the real stimulus will likely to occur if the signal that is used to detect a stimulating event (stimulus channel) is not a real stimulus artefact but most likely a pulse input to the stimulator to trigger it (with a delay).

J. Hold time after trigger: ms

The user has the possibility to indicate the length of time period during which the program will not look for a stimulus artefact. This feature prevents the detection of false triggers in a specific time window. It is recommended to indicate a period of time which covers almost the time interval between the application of the triggers during the data acquisition.

K. Fixed trigger points [y/n]:

In the case when no signal (channel), which can be used for triggering the averaging procedure, has been recorded, and when, and only when, the stimulus was applied at a constant frequency, it is possible for the user to indicate exactly when these trigger spikes are occurring. For using this feature, the user has to answer yes to the current question and will also have to indicate choices at the next two questions.

L. Time of first trigger point: ms

First the user has to indicate at what time within the file the first trigger point is occurring. This information can be obtained while in the "DISPLAY OF RAW DATA" screen. When using the display of the cursors (crossed hairs), the user is informed about the absolute time position of the two cursors, after pressing the right button of the mouse.

M. Time interval between trigger points: ms

After indicating the position of the first trigger point, the user has to indicate the time interval between each trigger point. This is possible only in cases where the stimulus is applied at a constant frequency. The value of this time interval can be obtained, as for the time of first trigger point, while in the "DISPLAY OF RAW DATA"

screen. After pressing the right button, this number will appear on the screen. For more details, see section B.4.1 'Crossed hair cursors'.

Initial values information

N. Evaluation of initial values [y/n]:

There is a possibility for the user to obtain a mean value for other signals than the signal that is used to distribute the spike-triggered sample in different intervals. This mean value will be calculated for a time period which is identical to the one used to bin the samples. A 'yes' answer will enable the program to calculate the initial values for as many channels as indicated below. The list of channels has to be given in the second next question. These values will be displayed on the screen when activating the RANGE function (see section B.7.13), and on the page when printing the averaged traces with the HARDCOPY function (see section B.7.9).

O. Number of channels:

Indicate the number of channels for which the initial values (mean of the signal prior to the occurrence of the stimulus) have to be evaluated. This does not include the channel used to distribute the spike-triggered samples in the correct amplitude interval. The time period for which the mean will be evaluated is the same then the one indicated at points (C) and (D).

P. Channels are:

Indicate the channel numbers for which you want the program to evaluate initial values. It is not necessary to mention the channel which is used to distribute the spike-triggered samples into different intervals, initial values for this particular channel are automatically calculated..

B.6 PHASE AVERAGING

B.6.1 An overview

This function averages the data based upon the time of occurrence of an event. For example, it is possible to average reflex responses by grouping them according to the time within a rhythmic cycle at which a stimulus is applied. A set of parameters can be selected by the user, providing substantial flexibility in the averaging procedure. Default parameters are already selected. Details about these parameters are given below.

B.6.2 Parameters

The following is a list of all the parameters which has to been determined by the user in order to average the data based on the time of occurrence of the stimulus. Some of these parameters need to be determined only if a previous parameters has been selected. In the case that a parameter is not required, it is not listed on the screen. All parameters have been attributed a default value. The user can change any of these values. The user can also save a list of parameters which can be retrieved later, as for similarly averaged data from different files.

A modification can be done by typing the letter (green) preceding each parameter line. In typing 's', the user can save the current set of parameters for a later use. The same way, in typing 'r', the user can retrieve a parameter file which had been previously saved. Each of these requests have to be followed by <enter>. When satisfied with the parameter selections, the user can type 'q' to quit.

Note that these parameter values are kept as determined by the user as long as they are not changed, or that the user does not quit the program (return to DOS).

Interval information

A. Number of phases [1-16]:

Number of bins in which the spike-triggered samples will be distributed.

B. Use undisturbed for full cycle averages [y/n]:

An average of the data over a full step or movement cycle is performed by the program automatically. If 'y' is answered at this question, the program will only use the undisturbed steps or cycles to calculate the average. Otherwise, all the cycles will be used.

Stimulus information

C. Stimulus channel:

Input the number of the channel which will be used as the stimulus channel. The program will search on the signal recorded in this channel for a threshold crossing (fixed at the next point).

D. Stimulus threshold: (unit)

The user has to indicate what is the threshold (expressed in the units used by the channel selected in the previous point) at which the program will detect a stimulus. The program will mark the position of a trigger when the signal recorded in the stimulus channel crosses above the threshold value. No other trigger position will be marked until the signal goes below the threshold value. If the user would prefer marking a

trigger position when the signal crosses below the threshold value rather than above, it is possible, but indirectly, by inverting the stimulus signal indicating a negative gain value in the characteristic parameter for this specific channel. In any case, a threshold value can be evaluated by the user in the Raw Data Display Screen, using the mouse cursor to find the appropriate threshold.

E. Distance of real trigger after artefact: ms

In the case when the stimulus artefact used to trigger the spike-triggered averaging came much earlier than the actual electrical of mechanical perturbation, it may be appropriate to move the position of this artefact forward to get closer to the real time of application of the stimulus. This will affect the position of the period of time used to estimate an amplitude level for the purpose of distributing the spike-triggered samples in different intervals. This increase time difference between the stimulus artefact and the application of the real stimulus will likely to occur if the signal that is used to detect a stimulating event (stimulus channel) is not a real stimulus artefact but most likely a pulse input to the stimulator to trigger it (with a delay).

F. Hold time after trigger: ms

The user has the possibility to indicate the length of time period during which the program will not look for a stimulus artefact. This feature prevents the detection of false triggers in a specific time window. It is recommended to indicate a period of time which covers almost the time interval between the application of the triggers during the data acquisition.

G. Fixed trigger points [y/n]:

In the case when no signal (channel), which can be used for triggering the averaging procedure, has been recorded, and when, and only when, the stimulus was applied at a constant frequency, it is possible for the user to indicate exactly when these trigger spikes are occurring. For using this feature, the user has to answer yes to the current question and will also have to indicate choices at the next two questions.

H. Time of first trigger point: ms

First the user has to indicate at what time within the file the first trigger point is occurring. This information can be obtained while in the "DISPLAY OF RAW DATA" screen. When using the display of the cursors (crossed hairs), the user is informed about the absolute time position of the two cursors, after pressing the right button of the mouse.

I. Time interval between trigger points: ms

After indicating the position of the first trigger point, the user has to indicate the time interval between each trigger point. This is possible only in cases where the stimulus is applied at a constant frequency. The value of this time interval can be obtained, as for the time of first trigger point, while in the "DISPLAY OF RAW DATA" screen. After pressing the right button, this number will appear on the screen. For more details, see section B.4.1 'Crossed hair cursors'.

Initial values information

J. Evaluation of initial values [y/n]:

There is a possibility for the user to obtain a mean value for all selected signals This mean value (corresponding to a background value at time of stimulus application) will be calculated for a time period as indicated below. A 'yes' answer will enable the program to calculate the initial values for as many channels as indicated below. The list of channels has to be given in the second next question. These values will be displayed on the screen when activating the RANGE function (see section B.7.13), and on the page when printing the averaged traces with the HARDCOPY function (see section B.7.9).

K. Number of channels:

Indicate the number of channels for which the initial values (mean of the signal prior to the occurrence of the stimulus) have to be evaluated. The time period for which the mean will be evaluated is determined below, at points (M) and (N).

L. Channels are:

Indicate the channel numbers for which you want the program to evaluate initial values.

M. Delay before stimulus artefact: ms

The evaluation of the initial values will be done over a time period which normally is preceding the stimulus artefact. The user is required to indicate at what point in time (how many milliseconds prior to the stimulus artefact) this average has to start to be calculated.

N. Length of time used to determine the initial values: ms

How many milliseconds are necessary from the time indicated in (M) to evaluate the mean value of a signal just before the application of a stimulus. It is suggested to allow for more time for an EMG mean than for a force or a length signal.

B.7 AVERAGED DATA FUNCTIONS

Most of the functions listed below can be used from both Amplitude and Phase averaging procedure. Although a few functions are available only for the Phase averaging procedure. These functions are labelled with a superscript '1' on Fig. B.1. To select a function, just enter a green letter and press <enter>.

B.7.1 AVERAGE

Once the traces are overlaid the average can be displayed in a white trace, superimposed onto all the individual sample traces.

B.7.2 CLS&AVE

In this case, the screen will be clear of all the individual sample traces and the average only will be displayed in white, for each bin.

B.7.3 DISCRETE

This function will calculate the coefficients for a discrete Fourier analysis. The user will be prompted to enter some parameters in order to perform the analysis. It is also possible to reconstruct the signal, from the calculated coefficients, in order to verify the accuracy of the analysis (often dependent on the choice of parameters).

B.7.4 SPECTRAL

This function allow the preparation of a signal for a spectral analysis. The program does not have the ability to actually perform this spectral analysis. It is only able to prepare a file which will then be used by another C program.

B.7.5 RESCALE

This RESCALE function works the same way as for the raw data display screen. The display amplitude can be adjusted with the RESCALE function. After selecting RESCALE the program will prompt (again in the bottom right) for an upper and lower limits. These limits correspond to the upper and lower limit (in the channel unit) you would like displayed in the window provided for that channel.

B.7.6 AMPLIFY

This function will amplify each individual average (for each bin) by multiplying every point by an amplification factor given by the user. This factor has to be a whole number (integer).

B.7.7 SMOOTH

A data smoothing routine was written which will lowpass filter the data. The SMOOTH option will prompt the user for the number of points in the window used by the moving average. If 20 points are chosen the data point is averaged with 10 points preceding it and 10 points following it. In this way no phase shift occurs. The original average is saved and the filtered trace is displayed on the screen. Therefore the original average can be retrieved at any time by selecting SMOOTH and then 'r' for restore. Remember that smoothing only occurs on the original data. Therefore if you filter once with a window of 10 points and filter immediately after with a window of 20 points you do not have filtered with 30 points!

B.7.8 HISTOGRAM

The program can build a histogram for looking at time of occurrence of some specific spiky events. The histogram can be applied to amplitude averaged data, or phase averaged data (both undisturbed and disturbed averages). The number of bins in the histogram will depend on the data chosen. Some flexibility is also given to the user for designing the histogram.

B.7.9 HARDCOPY

Hardcopy output can be obtained by selecting the HARDCOPY function in the menu listed at the top of the screen. The HARDCOPY function plots the averaged traces to an HPGL compatible plotter in HPGL mode.

B.7.10 EXPORT

An ASCII output of all the averaged traces can be obtained by selecting EXPORT from the menu. EXPORT will first ask if the user wants the disturbed or undisturbed data store in an ASCII, in the case that the phase averaging procedure has been used. EXPORT will also prompt for a file name in which to store the ASCII data. The extension **.MAT** will automatically be added to the filename given by the user (if the disturbed data is stored), otherwise, the extension **.CYC** will be used. The program stores the data in columns; the number of columns corresponds to the total number of bins plus one. The first column contains the data point of the time vector. The other columns contain the data, one column per bin, starting with the first bin. Every sampled point will be sent to the ASCII file.

Remember, the ASCII file will be stored in the current directory if you do not indicate a new path.

B.7.11 *.PLT

The *.PLT function works the same way as for the main menu. This function allows the user to save the content of the screen in a file compatible with the software CORELDRAW (graphic drawing file). The user will be prompted to input a name for the file, without the extension. The extension **.PLT** will be automatically added to the filename provided by the user. The user have the choice to send the individual phases, or the full undisturbed cycle in the case of a phase averaging. In the former case, as well as for the normal averaging mode, every bin traces will be send to the graphic file, as they appear on the screen.

B.7.12 HISTO_EXP

The content of the histograms, calculated with the HISTOGRAM function can be saved in an ASCII file by using HISTO_EXP. The user will be prompted to enter a file name, without an extension. The program will automatically append the extension **.HIS** if the disturbed data histograms are stored, and the extension **.UND** if the undisturbed data histogram is stored. This function should be call immediately after the results from the HISTOGRAM function are displayed on the screen.

B.7.13 RANGE

This subroutine requires as input a starting and ending point in the average trace and then uses that "range" to find a number of values for the user. The total number of points in the trace are displayed in the bottom right corner of the screen. The start and end of the range are chosen relative to the total number of points in the trace. The range is then displayed in magenta while the rest of the average is displayed in white. Various values can be obtained with the RANGE function such as mean value between two points, positive and negative peaks, peak-to-peak values, etc.

B.7.14 STDERR

A one standard error margin can be evaluated over the period selected in the RANGE function. This will display two flat lines at +1 SE and -1 SE.

B.7.15 FULL CYC

The undisturbed average, calculated for every channels during the phase averaging procedure can be displayed on the screen with the FULL CYC function. The user will be asked to type in each channel that needs to be displayed on the screen. Once the user has listed all the desired channel numbers, 'q' needs to be typed to exit the FULL CYC function.

B.7.16 PLOT CYC

This function will send the averaged undisturbed traces to the printer (HPGL compatible), when the phase averaging procedure has been used.

B.7.17 SUBTRACT

It is possible to subtract either the initial value, the undisturbed data, or a trend from the disturbed averaged traces. This can also be used with amplitude averaging, without asking for subtracting the undisturbed data. The trend is calculated within the RANGE function.

B.7.18 STAT DUR

Statistics on the duration of full steps, stance and swing phases can be displayed on the screen with the STAT DUR function. These statistics include a number of samples, and the duration mean and standard deviation for each disturbed bin. Note that this data is automatically stored in an ASCII file, with extension **.DUR**.

B.7.19 QUIT

This will send you back to the raw data display screen from where you can ask for a different averaging, or for the same one with different averaging parameters. A complete quitting of the program can be done once back with the raw data display screen.

B.7.20 CHAN#

Typing a channel number will display on the screen all the samples for a specific signal, distributed in the appropriate bin.

HAVE FUN!